JPRS 71491

18 July 1978

USSR

TRANSLATIONS ON USSR SCIENCE AND TECHNOLOGY

BIOMEDICAL AND BEHAVIORAL SCIENCES

No. 36

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BIBLIOGRAPHIC DATA SHEET	1. Report No. JPRS 71491	2.	3. Recipient's Accession No.
4. Title and Subtitle TRANSLATIONS ON USSR SCIENCE AND TECHNOLOGY BIOMEDICAL AND BEHAVIORAL SCIENCES, No. 36			5. Report Date 18 July 1978 6.
7. Author(s)			8. Performing Organization Rept. No.
9. Performing Organization Name and Address Joint Publications Research Service 1000 North Glebe Road Arlington, Virginia 22201			10. Project/Task/Work Unit No. 11. Contract/Grant No.
12. Sponsoring Organization Name and Address As above			13. Type of Report & Period Covered 14.

15. Supplementary Notes

16. Abstracts

The report contains information on aerospace medicine, agrotechnology, bionics and bioacoustics, biochemistry, biophysics, environmental and ecological problems, food technology, microbiology, epidemiology and immunology, marine biology, military medicine, physiology, public health, toxicology, radiobiology, veterinary medicine, behavioral science, human engineering, psychology, psychiatry and related fields, and scientists and scientific organizations in biomedical fields.

17. Key Words and Document Analysis. 17a. Descriptors

USSR
Aerospace Medicine
Agrotechnology
Biology
Botany
Epidemiology/Immunology
Human Engineering
Marine Biology
Medicine
Microbiology
Physiology
Psychology/Psychiatry
Public Health
Radiobiology
Toxicology
Veterinary Medicine

17b. Identifiers/Open-Ended Terms

17c. COSATI Field/Group 2, 5E, 5J, 6, 8A

18. Availability Statement Unlimited Availability Sold by NTIS Springfield, Virginia 22151	19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 85
	20. Security Class (This Page) UNCLASSIFIED	22. Price PC A05

JPRS 71491

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BIOCHEMISTRY

PROTECTIVE EFFECTS OF A SERIES OF NEW PYRIDINIUM DERIVATIVES AGAINST INHIBITION OF ACETYLCHOLINESTERASE BY FLUOSTIGMINE

Berlin DIE PHARMAZIE in English No 2/3, 1978 pp 120-121

[Article by J. Faff, W. Raszewski and S. Rump]

[Text]

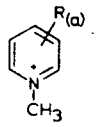
The protective effects of a series of new pyridinium derivatives against inhibition of acetylcholinesterase (AChE) by fluostigmine was studied in vitro on human erythrocytes. It was stated that some of these compounds exhibit protective action for AChE against inhibition by fluostigmine. The best protective index had N-methyl-4-[γ-pyridyl]-pyridinium iodide.

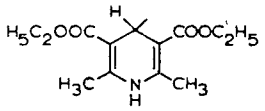
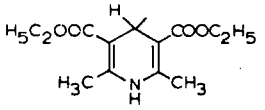
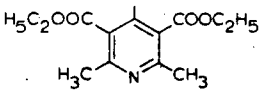
1. Introduction

The treatment of poisoning with organophosphates involves the use of a cholinolytic drug and an oxime. The effectiveness of the latter in reactivating inhibited acetylcholinesterase (AChE) depends upon the kind of organophosphate used. This is due to the various times consumed in the so-called "ageing" of the inhibited AChE (dealkylation of the organophosphorus component of the phosphorylated enzyme) which leaves the enzyme resistant to reactivation. With some organophosphates, even a very rapid administration of oximes was unsuccessful [1, 3, 5].

One of the aims in the search for new antidotes is to find agents whoch would protect AChE against organophosphates. It was reported that certain oxime-free bispyridinium salts do exhibit an antidotal effect and this effect is not based on enzyme reactivation [6, 7].

Table 1 Chemical Structure of Tested Pyridinium Derivatives



Compound	a	R
PAN-W-29	–	–
PAN-W-40	2	$CH\text{-}(CH_3)_2$
PAN-W-33	2	$CH{=}N\text{-}C(CH_3)_3$
PAN-W-34	4	$CH{=}N\text{-}C(CH_3)_3$
PAN-W-35	4	$CH{=}N\text{-}CH(CH_3)_2$
PAN-W-31	3	$C(CH_3)_3$
PAN-W-53	4	$C(C_2H_5)_3$
PAN-W-43	4	$CH(CH_3)_2$
PAN-W-41	4	C_2H_5
PAN-W-49	2	C_6H_5
PAN-W-44	4	$O\text{-}C_6H_5$
PAN-W-46	4	$S\text{-}C_2H_5$
PAN-W-47	4	$S\text{-}CH(CH_3)_2$
PAN-W-48	4	$S\text{-}C_6H_5$
PAN-W-51	4	
PAN-W-5	4	
PAN-W-4	2	
PAN-W-50	4	

Our investigations have been made to study the protective effects of a series of new, originally synthesized, monopyridinium derivatives without oxime radicals, against inhibition of AChE by an organophosphate.

2. Results and Discussion

Acute toxicity of tested drugs, their inhibitory effect on AChE and their protective effect on AChE against inhibition by fluostigmine are shown in Table 2. The best protective index was obtained with compounds PAN-W-51, PAN-W-40 and PAN-W-34.

Table 2 Acute Toxicity, Inhibitory Effect (pI_{50}) and Protective Effect for AChE Against Inhibition (pE_{50}) with Fluostigmine (Conc. $1.4 \cdot 10^{-7}$ M)

Compound	LD_{50} [Mol/kg · 10^{-5}]	Pi_{50}	pE_{50}	PI*
PAN-W-29	222.2 (152.8–277.7)	2.9586	2.9914	0.9890
PAN-W-40	58.3 (40.1– 84.7)	2.9830	3.3979	0.8778
PAN-W-33	80.6 (59.0–109.6)	3.3010	3.3979	0.9714
PAN-W-34	176.4 (140.0–221.1)	2.6192	2.9830	0.8780
PAN-W-35	337.8 (247.6–460.4)	2.9747	3.0706	0.9687
PAN-W-31	103.6 (64.9–155.3)	2.6990	3.0223	0.8930
PAN-W-53	21.6 (14.5– 31.4)	2.9666	2.9666	1.0000
PAN-W-43	65.7 (47.2– 91.0)	3.3188	3.3010	1.0053
PAN-W-41	98.4 (61.8–156.6)	3.3010	2.9957	1.0119
PAN-W-49	33.4 (29.4– 37.9)	3.0000	3.0000	1.0000
PAN-W-44	24.1 (21.7– 29.1)	2.5229	2.4815	1.0166
PAN-W-46	35.3 (28.0– 45.2)	3.1549	2.9626	1.0649
PAN-W-47	28.8 (26.0– 31.8)	3.0000	2.9747	1.0085
PAN-W-48	17.2 (15.6– 19.3)	2.4202	2.2218	1.0892
PAN-W-51	89.3 (78.0–102.6)	2.5850	2.9987	0.8260
PAN-W-5	92.1 (62.7–113.8)	3.0000	3.0458	0.9849
PAN-W-4	58.3 (33.7– 88.4)	2.9957	2.6090	1.1482
PAN-W-50	91.1 (76.1–108.8)	2.3979	2.3979	1.0000

* Protective index = $\frac{pI_{50}}{pE_{50}}$

The pE_{50} data of tested drugs were plotted against pI_{50} and compiled in Fig. 1. These results indicate quite close correlation between the two phenomena under examination. Correlation coefficients ($r = 0.72$) were statistically significant ($P < 0.05$).

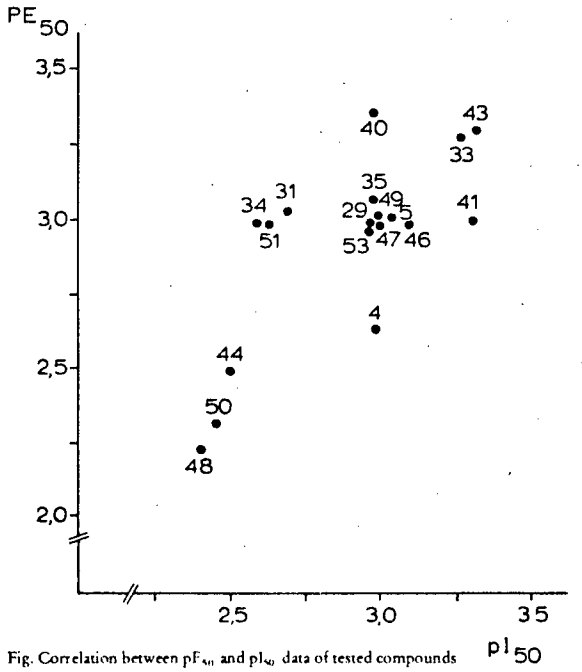


Fig. Correlation between pE_{50} and pI_{50} data of tested compounds

It shown by many authors that some pyridinium derivatives, like other compounds posessing quaterary nitrogen in their molecule, exhibit protective effect for AChE against organophosphates. However, many of these compounds are also reversible inhibitors of AChE.

The mechanism of that effect could be explaines as – competitive reaction of the drug with ACh for the anionic site of the enzyme, – protection of esteratic site following transient bond between the drug and anionic (or other) site of the enzyme, or – change in reactivity of esteratic site due to the reaction of the drug with the enzyme.

The protective effect of drugs tested was closely dependent on their ability to inhibit AChE, which was pointed out from the correlations between pI_{50} and pE_{50} data. For all drugs tested the ratio between pI_{50} and pE_{50}, called the protective index (PI), was near 1. Relatively the best PI value was obtained when compound PAN-W-29 was substituted with pyridyl (PAN-W-51). However, the methylation or esterification of pyridinium substituent also increased the PI value. Substituting pyridinium with the phenyl ring in 2 position (PAN-W-49) only slightly increased pE_{50}. However, phenyl substition in 4 position attached through oxygen (PAN-W-44) or sulphur (PAN-W-48) decreased the protective potency. Compounds having sulphur atom in the substituent (PAN-W-46 and PAN-W-47) had weaker protective potency and higher acute toxicity than their analogues with alkyl radicals (PAN-W-41 and PAN-W-43).

3. Experimental

3.1. Acute Toxicity

Each compound was tested on male Swiss mice with an average body weight of 22 g. The LD_{50} was determined for 24 h observation after i. p. administration of the drug by the method of moving averages and interpolation described by *Thompson* [8] with additional tables prepared by *Weil* [9].

3.2. Inhibitory Effect on AChE

The source of AChE were human erythrocytes, centrifuged and washed with saline and diluted with 0.01% saponin and than with 0.04 M phosphate buffer (pH = 7.6) to the final concentration of 20 μl/ml. Aqueous solutions of the tested drugs in various concentrations were added to the enzyme solution in a ratio 1 : 1 in a bath at 37 °C. Within 5 min an aqueous solution of acethylcholine chloride was added and the activity of AChE was measured by the method described by *Hestrin* [4]. Concentration of acetylcholine in the begining of the reaction was $2 \cdot 10^{-3}$ M. pI_{50} (negative logarithm of the concentration of drug which inhibited 50% of the enzyme) were determined by the graphic method.

3.3. Protective Effects an AChE

Human erythrocytes, three times washed with saline and centrifuged, were tenfold diluted with 0.01% saponin. One ml of this solution was placed to the bath at 37 °C. Then 0.5 ml of the tested drug dissolved in 0.04 M phosphate buffer (pH = 7.6) was added. Within 5 min 0.5 ml of the solution of fluostigmine in 0.05 M phosphate buffer (pH = 7.6) was also added. Final concentrations of the tested drugs in experimental solutions were: $2.5 \cdot 10^{-1}$ M, 10^{-1} M, $2.5 \cdot 10^{-4}$ M and 10^{-4}; the concentration of fluostigmine was $1.4 \cdot 10^{-7}$ M. The resulting solution was incubated at 25 °C for 30 min and after that the activity of AChE was measured using the method described by *Ellman* and co-workers [2].

The protective enzyme activity was expressed as a percentage using the formula

$$\frac{Ap - Ai}{Av - Ai} \cdot 100$$

Av = AChE activity in the begining of the experiment
Ai = AChE activity after the administration of the inhibitor (fluostigmine)
Ap = AChE activity after the administration of protective agent and inhibitor

pE_{50} (negative logarithm of the concentration of drug which protected 50% of AChE against inhibition) was calculated graphically. The protective index (PI) was expressed as a relation of pI_{50} to pE_{50}.

3.4. Substances Used

Tested drugs with their chemical structures are listed at Table 1. They were synthesized originally by Prof. Dr. P. Nantka-Namirski with collaborators (Institute of Organic Chemistry, Polish Academy of Sciences). Fluostigmine (diisopropyl phosphorofluoridate; DFP) of 95% purity was obtained from Dr. Witek (Institute of Organic Industry, Warsaw). Acetylcholine chloride (Sigma), acetylthiocholine iodide (Chemapol) and S.S-dithio-bis[2-nitrobenzoic]acid (Sigma) were of an analytical grade.

Acknowledgement: This work was supported in part by the grant from the Polish Academy of Sciences under the research project N°. MR-I-12.2.3.18.

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Received May 9, 1977

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INDUSTRIAL MICROBIOLOGY

FRIENDLY COMPETITION IMPROVES PRODUCTION

Moscow MEDITSINSKAYA GAZETA in Russian 17 Feb 78 p 1

[Article by Ya. Men', Belgorod]

[Text] For 14 years the shop collectives of the Belgorodskiy vitamin combine named "The Fiftieth Anniversary of the USSR" and the Bolokhovskiy chemical combine for synthetic semi-finished products and vitamins have been competing in the output of vitamin C. This helps them raise the production indices and improve the technology.

During the first days of February, Belgorodians received a delegation from the related enterprise, which had arrived to review the totals for the past year. The competitors successfully coped with their socialist obligations. The Belgorodians had produced over and above the plan, and the Bolokhovtsi produced still more. But in economication of raw materials and energy resources, it turned out the other way around. The combine workers of The Fiftieth Anniversary of the USSR noticeably outstripped their rivals.

And, according to the majority of basic indices, the workers from the Bolokhovskiy combine improved everything. First place was awarded to them.

By tradition, a collective agreement was drawn up at the meeting between the two enterprises about socialist competition in 1978.

CSO: 1870

MICROBIOLOGY/IMMUNOLOGY

UDC: 575.224.232.3.04(047)

INVOLVEMENT OF R PLASMIDS IN TRANSFER OF CHROMOSOMAL GENES

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5, 1978 pp 3-10

[Article by I. V. Domaradskiy, All-Union Scientific Research Institute of Protein Biosynthesis, submitted 1 Nov 77]

[Text] We already know of the capacity of resistance factors (R plasmids) to participate in the transfer of chromosomal genes. Sugino and Hirota [72] were the first to call attention to this. Subsequently, the findings of the Japanese authors were confirmed and expanded [26, 40, 63]. Ultimately, some interesting facts were obtained, which characterize R plasmids as rather unique genetic systems. We shall try to sum them up:

1. Most R plasmids of the wild type are present in cells in a repressed state, as a result of which the incidence of transconjugants in crosses with strains carrying such plasmids is usually a function of derepression of R plasmids.

2. Transfer of chromosomal markers, induced by R plasmids, always begins with the transfer of the R plasmid to a recipient; no exceptions have been demonstrated thus far.

3. All, or virtually all transconjugants obtained by means of R plasmids acquire the capacity to become donors.

4. Most R plasmids referable to different incompatibility groups are capable of transferring chromosomal markers.

5. Some R plasmids can begin the transfer of markers from different sites of the chromosome [35, 40], while others have fixed transfer sites [37, 63].

6. Many R plasmids impart to bacterial cells the capacity for restriction and modification of foreign DNA (hsp genes), and this capacity is inherent in I-like R plasmids [6, 76]; to the best of our knowledge, only one R factor of the F type, with hsp, has been discovered [34].

Another quite important distinction of R plasmids, which we shall discuss in greater detail, is that their change from an independent to integrated state is an exception rather than the rule [6]. At the same time, the F factor can be inserted readily in the chromosome as a result of reciprocal recombination, i.e., it is one of the typical episomes [23]. Let us stress that the capacity to induce chromosomal transfer at a high frequency is considered typical of expressly integrated plasmids.

Direct evidence of the possibility of insertion of R plasmids in a chromosome was obtained quite recently by the method of intrative suppression of dnaA mutations [58]. As shown by Moody and Runge [57], F-like R and Col factors are among plasmids that determine integrative suppression; there are indications in the work of Datta and Barth [29a] that some I-like R plasmids have this capacity. It was also established that many "revertants" appearing as a result of integration of R or ColV2 factor behave like genuine Hfr strains. Hence, at least some R plasmids are indeed incorporated in the chromosome and cause appearance of clones with different start and direction of chromosomal transfer. To what then is related the transfer of chromosomes in other cases, in particular, in the case of plasmids that are incapable of inducing even integrative suppression? Unfortunately, no unequivocal answer can be given to this question for the time being.

Although we have discussed only R plasmids, since expressly they are the subject of our survey, it should be stressed that all or virtually all of the distinctions of R plasmids as genetic systems are also inherent in conjugative colicinogenic factors. In particular, many of them cannot be viewed as episomes, in spite of the fact that they are capable of transfering chromosomal markers (ColI is a typical example) [53, 70]. For this reason the question of mechanism of this transfer is of general significance.

It should be recalled that chromosome transfer by most plasmids depends on the rec system of their hosts [20, 24, 56]. With the use of donor recA the frequency of transfer of chromosomal markers drops sharply; however, not to zero [24]. It is believed that this low ("residual") level of transfer (of the order of 10^{-8}) is unrelated to retention of rec system function, since loss of polarity is observed with transfer of chromosomal markers from a recA donor. It depends on some other, unusual mechanism. The same mechanism is apparently also involved in transfer of genes by means of R plasmids, where it is also unrelated to the function of rec systems [23, 36, 56].

There are two factors that point to the existence of an unusual mechanism of genetic transfers: in the first place, induction of chromosomal transfer, which follows exposure of F^+ culutres to ultraviolet light, is unrelated to appearance of Hfr donors, although it is controlled by rec functions of bacteria [32]; in the second place, some F- and I-like plasmids can transfer nontransmissible plasmids in the absence of an overt structural link between them.

The uniqueness of the hypothetical mechanism of genetic transfer does not preclude the fact that there is some specificity to it. In the case of

transfer of chromosomal markers, it is manifested by the fact that some R plasmids have specific "recognition" sites [37, 63]. When nontransmissive plasmids are mobilized, specificity may be manifested by the choice of a "partner," i.e., a conjugative conductor-plasmid [36, 65].

Referring to the question of chromosomal transfer by means of sex factors, G. Meynell [6], citing the works of Curtiss [28] and Curtiss and Stallions [29], wrote: "A direct comparison of incidence of recombinations to the number of stable Hfr clones shows that they can explain only 15% of the formed recombinants." From this, it could be concluded that the Hfr state is not the only prerequisite for chromosomal transfer from F^+ cultures; however, the marked dependence of transfer on rec systems of the host is against such a conclusion. We consider more warranted the hypothesis that about 85% of the Hfr clones are unstable, which is indeed the case in F^+ cultures [16, 28]. Evidently, the same occurs with most R plasmids; it is not possible to catch the moment of integration, and one has to determine that it occurred indirectly on the basis of the end result, i.e., appearance of chromosomal markers in transconjugants. Moddy and Runge [57], Moody and Hayes [56] voiced the thought that I-like plasmids (and, perhaps F-like ones in the case of "residual" transfer--I. D.) can integrate stably by means of reciprocal recombination without subsequent effective mobilization of the chromosome. Incidentally, these authors do not insist on their hypothesis.

The second hypothesis dealing with transfer of chromosomal markers by "non-episomal" plasmids is based on the assumption that nonreciprocal recombination is possible [56, 57]. In this case, recombination can take place without physical exchange of DNA fragments, by means of random copying with a changeable template. True, the model of random copying does not hold up to criticism; however, it does explain, quite satisfactorily, the mechanism of nonreciprocal recombination. Another explanation of the mechanism of non-reciprocal recombination [56] is based on the results of experiments dealing with induction of chromosomal transfer by the sex factor with exposure to ultraviolet light [32]. In the opinion of Evenchik et al., binding of the chromosome and plasmid occurs, in this case, only at the expense of one DNA strand. Such a structure can be transmitted through conjugation, but it is incapable of more than one replication cycle.

Data pertaining to the presence of tfa genes in repressed plasmid R1 may be rather important to interpretation of all aspects of the problem of transfer of chromosomal markers by R plasmids [36]. Loss of tfa genes is associated with arrested transfer of chromosomal markers at a high incidence, but does not affect transmission of the plasmid. Detection of similar genes in other R plasmids and identification of molecular mechanisms that determine them is a job for the future. In our opinion the results of the experiments of Coetzee are also interesting [25]: An R plasmid, incapable of transferring chromosomal markers, acquired this capacity after recombination with a plasmid which also did not elicit transfer on its own. Finally, summation of properties of RP1 and FP2 plasmids merits attention [59].

It is not by chance that investigators are interested in the capacity of R plasmids to transfer chromosomal markers. When R and F plasmids were compared in this respect, advantages were found more often in the former, not to mention the fact that some R plasmids transfer chromosomal markers more efficiently than F plasmids [71]. The main advantage of R plasmids is that they can be transmitted to many bacteria. This opens up the prospect of exchanging genetic information between remote species of microorganisms, for example, transmission of R plasmids from E. coli to obligate anaerobes [17].

Efforts to cross different species of microorganisms were undertaken soon after the discovery of the conjugation phenomenon, mediated by sex factors. Some were successful. It was shown that representatives of various genera of intestinal bacteria, in particular shigella, salmonella, serratia, proteus, yersinia and even vibrions, may serve as recipients. Many of them, also received chromosomal markers, in addition to the sex factor. In some cases, the transconjugants themselves began to emerge in the role of donors of genetic information, transmitting to new recipients substituted sex factors or large fragments of their own chromosomes. However, limitations to such crosses also began to be demonstrated. Let us mention some of them:

1) It is difficult to predict the outcome of a cross: other conditions being equal, some species of a given bacterial genus yield transconjugants and others do not.

2. Even if the crosses are fertile, the transconjugants are not always donors, or else they may be very "discriminating" in the choice of partners.

3. The range of hosts is relatively narrow, and it is usually limited to representatives of the family of enterobacteria.

It cannot be stated that crosses mediated by R plasmids are totally wanting in these flaws. Here too, the outcome of the cross is not always clear, while new hosts of R plasmids are not necessarily donors of genetic information. But these limitations are not as marked as in the case of sex factors. Moreover, the range of hosts is unquestionably broader, which makes it possible to circumvent other limitations (see below).

R plasmids of B. aeruginosa, referable to incompatibility group P merit special attention [75]. At the present time, over two dozen R plasmids have been found in strains of B. aeruginosa [43]. However, we shall only discuss the two that have been studied more than the others. The first, RP1 plasmid (it is also RP4 [42]) has an exceptionally wide range of secondary hosts (see Table). In addition, it is capable of intergeneric transfer of chromosomal markers [4, 39]. The second plasmid, R68, or more precisely its R68.44 and R68.45 variants [35, 43], is remarkable, primarily as a carrier of chromosomal genes between strains of the PAO line of B. aeruginosa. The capacity to transfer rather large chromosomal fragments, equivalent in length to 10-30 min on the chromosomal map plotted by means of the FP2 plasmid, is an important distinction of R68 plasmid variants. As a result, it is possible to map the

the region of the chromosome of B. aeruginosa, situated distal to the 40th min, which had not yet been feasible with the use of FP2 or FP39 plasmids.

Bacteria that are secondary hosts of R plasmids of the P1 group

Genus	Source*
Escherichia	73
Salmonella	60
Shigella	21, 60
Proteus	60
Erwinia	22, 33
Vibrio	60
Aeromonas	61
Chromobacterium	31
Neisseria	60
Acinetobacter	60
Myxococcus	62
Azotobacter	60
Pseudomonas	46, 52, 60
Rhizobium	12, 31, 64
Agrobacterium	31
Caulobacter	10
Rhodospirillum	60
Rhodopseudomonas	60

*References to two or more sources indicates that several species of a given genus of bacteria may be plasmid hosts.

The mechanism of transfer of chromosomal genes by both plasmids has not been definitively identified. However, in this case, there are more grounds to maintain that insertion thereof in the chromosome is a prerequisite for mobilization thereof. Unfortunately, the relevant data were not obtained on the main host, B. aeruginosa, but on E. coli; moreover, these data are not referable to group P plasmids as such.

Ingram et al. [44] discovered an RP1 variant, named RP1-1 (R18-1 in other works). The RP1-1 plasmid had lost determinants Km and Tc and only retained the capacity to control resistance to Am; at the same time, it became less "aggressive," no longer was transmitted to Proteus mirabilis, and was transmitted only at a low frequency to E. coli. At the same time, the RP1-1 plasmid was not eliminated with dodecylsulfate and did not form a satellite peak of DNA when centrifuged in a cesium chloride density gradient. This plasmid behaved similarly in E. coli cells, and in addition it lost transmissiveness in them. On the basis of these facts, it can be concluded that RP1-1 is integrated with the chromosome. Richmond and Sykes [66] obtained additional information about this phenomenon. These authors believed that if RP1-1 is indeed inserted in the E. coli chromosome, this should be confirmed by transmission of the Am determinant along with other chromosomal

genes situated next to the site of RP1-1 integration. It was possible to obtain such transmission by addition of F_{13} in cells and crossing them with an appropriate recipient. Experiments using the Rec^- recipient yielded new evidence of recombination of RP1-1 with the chromosome.

We indicated above that data pertaining to integration of group R plasmids apply only to one of the RP1 variants. From this point of view, interesting results were obtained in experiments that demonstrated chromosomal transfer by a whole RP1 plasmid [77]. Unfortunately, we cannot form an opinion about the details of this work, since only an abstract thereof has been published.

Let us say a few more words about the R68 plasmid. There are fewer direct reports of integration thereof than insertion of the R18 plasmid. Nevertheless, the probability of integration is not completely ruled out. Appearance of R68.33 and R68.45 variants is an indirect indication thereof [35, 43]. The variants differed from the parental R68 plasmid in another way, instability. Loss of R68.44 and R68.45 is inherent in both donor cells and transconjugants that received them along with chromosomal fragments. Perhaps, for expressly this reason no Hfr type strains of B. aeruginosa were obtained. Data to the effect that the incidence of conjugation did not increase serve as addi tional proof of the "recombinant" origin of R68.44 and R68.45.

We can find examples of successful use of R plasmids to impart fertility to bacterial species, in which their own sex factors or other transmissive plasmids were not demonstrated, in a number of works [10, 13, 47, 51, 62, 74, 75]. The results obtained warrant the belief that R plasmids can be used to map chromosomes, at least of some bacteria [78].

Unfortunately, when direct crosses are attempted between many species of bac teria with the usual donors of R plasmids the desired results are not always obtained, and this is related both to species-related "incompatibility" of donor and recipient, and distinctions of the plasmids themselves. Among the factors that could influence the outcome of a cross, let us distinguish the following three:

1) anatomical and physiological distinctions of superficial structures of bacteria cells. Apparently, the greater the differences therein in two given species, the lower the probability of conjugation between them. However, there is an exception to this rule [17], and in some cases the mechanism of plasmid transfer remains unidentified [62];

2) recipient cell enzymes are incapable of identifying the nucleotide sequence of plasmid promoters, and as a result some operons are not transcribed [30] (for the time being, we cannot furnish more detailed information about this);

3) the action of restrictases.

Data on the effect of heating cells on appearance of transconjugants serve as confirmation of the possibility of plasmid restriction in the cells of inadequate recipients [14, 17, 27]. Brief heating at 50-52°C has an adverse effect on restrictase activity [54] and renders cells capable of integrating plasmids that they previously did not accept.* The same is indicated by the increased recipient capacity of r^-m^+ mutants [27]. One would think that processes of restriction also determine instability of R plasmids of different species of microorganisms [22, 61].

In those cases where attempted direct crosses between different bacterial species do not lead to positive results, one has to resort to the "sluice" method, i.e., successive transmission of plasmids of one intermediate recipient to another, until a level of competence is reached in a new donor that would permit transfer of the plasmid to the desired bacterial species [46, 48, 52]. This approach is not without empiricism; however, it does merit a close scrutiny.

To what can we attribute the beneficial effect of "sluicing" [gating]? Most likely, in the course of successive transfer of R plasmids they undergo modification, which ultimately lowers the sensitivity of plasmids to the corresponding restrictases or removes obstacles to transcription.

However, the sluicing method is not the only one. The range of hosts of given plasmids can be widened by "mobilization" thereof by other plasmids. In such a case, the leading role is apparently referable to recombination processes, and this is confirmed in the work of Hedges and Jacob [38]. Incidentally, recombination processes are not mandatory for mobilization, as indicated by Jacoby [45]. True, in his experiments, a transmissible plasmid (sex factor) was involved in transfer of a plasmid that was not conjugative. The mechanism of such transfers has not yet been identified [6].

Olsen and Wright described an instance of change in plasmid properties in the direction of widening the range of their hosts [61]. These authors established that Aeromonas salmonicida can relatively readily accept R plasmids of different compatibility groups from E. coli and Pseudomonas (FII, I, P, W, N, X). The Aeromonas itself becomes a plasmid donor, although not all determinants are transmitted with the plasmids. However, there is another interesting consideration. Assuming that partial fragmentation of plasmids in Aeromonas cells could alleviate recombination thereof, the authors "designed" strains with plasmids pMG1 (group P2) and R6K (group I) or R192.7 (group FII), notable for a narrow spectrum of hosts. As a result, it was learned that when such strains are crossed they transmit at an appreciable incidence each of the pairs of plasmids to both E. coli and Pseudomonas; in turn, all transconjugants transmit "aggregated" plasmids to the new Pseudomonas strain. It must be stressed that this plasmid property was acquired only in Aeromonas cells.

*Analogous findings are made when B. aeruginosa strains are incubated at 43°C; they retain the Res^- phenotype for about 60 passages at a temperature of 37°C, which is the optimum for growth [41, 67, 68].

Before this, while in cells of the main hosts (Pseudomonas in the case of pMG1 and E. coli in the case of R6K and R192.7), they had not been transmitted to other bacterial species.

With reference to the question of interspecific (intergeneric) transmission of plasmids, we should like to pose the question: Is not the capacity to be transmitted to other bacterial species related to the presence of special, as yet unidentified genes in the plasmids? Such genes may determine the attitude of plasmids to systems of restriction and modification of new hosts or trigger additional mechanisms that transfer the plasmids into a foreign cell. Bearing the latter in mind, we could conceive of the existence of "competence" genes, the products of which somehow broaden the range of plasmid hosts. Such genes have been demonstrated in the sex factor and R plasmids [2]. True, they control synthesis of recombinant entrance stimulator in intraspecific conjugation. However, as far as we know, their influence on the outcome of interspecific crosses has not yet been investigated. In the opinion of Chernin [9], competence genes are situated in "the transmissibility factor (RFT or Δ particle) R of episomes." Bakanchikov and Lobanok [1] believe that the corresponding locus is beyond the boundaries of the sex factor tra operon.

What is the fate of chromosomal genes that are transferred by plasmids to new hosts? Evidently, the simplest situation is in the cases when chromosomal genes integrate in the plasmid, i.e., when substituted plasmids (type I and type II crossing-over [69]) are formed as a result of illegitimate recombination. Then replication and expression of chromosomal genes occur simultaneously with analogous processes in plasmid genes. The extent of structural homology of chromosomal DNA in the new host and DNA of chromosomal markers of the donor does not play a substantial role, since recombination between them is not required for "acceptance" and normal function of exogenotes. As an example, let us cite our own data, which showed that the pro and his genes of B. aeruginosa are contained in E. coli as part of the RP1 plasmid [4].

The size of the chromosomal fragments integrated in plasmids varies widely, and in the case of F' factors it even reaches one-fourth the length of the E. coli chromosome [8].

Low has described the conditions that enhance appearance of F' factors [50]. It is quite probable that analogous conditions are also requried for the formation of substituted F-like R plasmids.

The situation is different when chromosomal genes are transferred in interspecific crosses without visible linkage with plasmids. There is a comprehensive survey [11] dealing with this question, and we refer to reader to it. Here, we shall merely mention the most vivid distinctions of transconjugants that appear in such crosses. All of them can be put in one of two classes. The first is characterized by the fact that the transconjugants are homozygotes, i.e., they are formed as a result of recombination of donor chromosome fragments and corresponding loci of the recipient chromosome. The second class of transconjugants is characterized by partial diploidy, which is related to "conservation" of exogenotes. It should, however, be mentioned

that one cannot draw a clearcut line between these two classes; there are a number of intermediate forms or subclasses [see, for example, 18, 19, 55].

The second class of transconjugants is of particular interest. In the last few years, works began to be published indicative of the possibility of existence of exogenotes in an independent state [11, 19, 49, 55]. The impression is gained that the fragment of a foreign chromosome is transformed into a special nonconjugative plasmid capable of replication without a link with the host chromosome. We cannot rule out the possibility that expressly this explains the origin of plasmids that control degradation of different organic compounds.

From the practical point of view, it is interesting that genes transferred to recipient cells by R plasmids do not always become strictly controlled by the new host [39].

In conclusion, we should like to stress the following:

R plasmids are a convenient genetic tool, which expands appreciably the possibility of genetic exchange between microorganisms that are taxonomically far apart. This conjugation method, which is determined by R plasmids, is superior to methods of transduction and classical transformation [3]. Moreover, transfer of R plasmids opens the way for developing crossing systems in bacteria that have none. R plasmids are also convenient, when they cannot be transmitted via conjugation. This obstacle can be overcome by using isolated plasmid DNA, as had been done in the experiments with hay bacillus [5, 7, 31a]. As a result, recipients often become fertile. Nor can we rule out the possibility of transformation, by means of recombinant R plasmids with other genetic elements, for example Mu phage, as "inserts" [15], capable of compensating for some of the flaws of R plasmids as vectors.

All of the foregoing compels us to revise the views of R plasmids and pay special attention to them, in order to take advantage of their unique capabilities to solve problems of modern genetics. Investigation of R plasmids will help to avoid many "dead ends," and aid in faster development of molecular biology.

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UDC: 616.981.42-008.97:619]-078:576.851.42.06.083.3

PATHOGENICITY OF BRUCELLA ISOLATED FROM WILD AND GAME ANIMALS OF THE EXTREME NORTH OF THE USSR

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5, 1978 pp 46-48

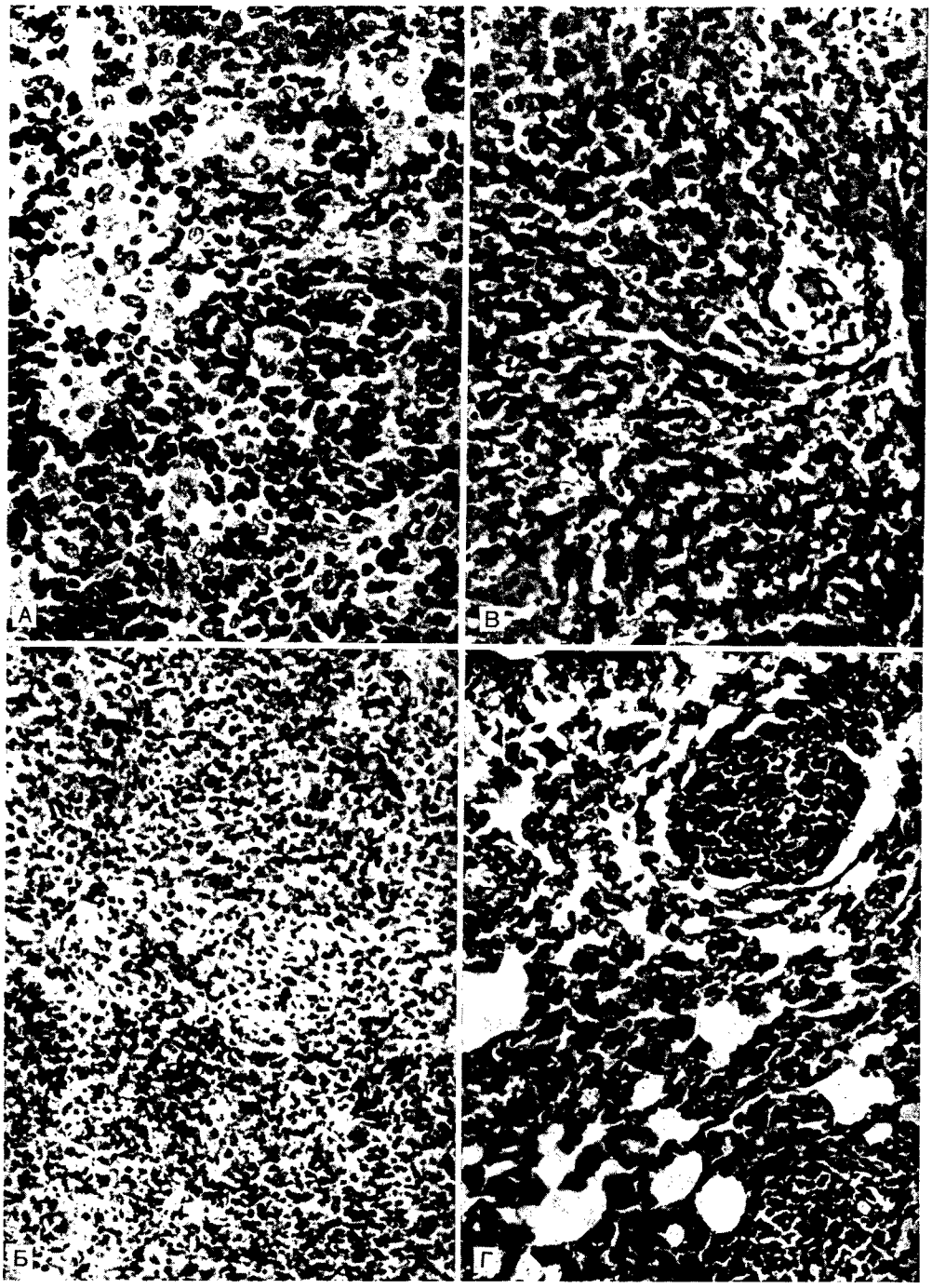
[Article by N. A. Grekova and L. V. Gorban', Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow, submitted 10 Jun 77]

[Text] In works published in recent years there is increasing accumulation of data concerning the existence of a stable endemic site of deer brucellosis in the Extreme North of the USSR [7, 8, 11, 13, 14, 15]. Circulation of brucella is observed not only among domesticated and wild deer, but other species of animals that are in direct or indirect contact with deer, the main source and reservoir of this infection. There are numerous data indicative of spontaneous infection of wild and game animals (wolf, Arctic fox, ermine, wolverine and others) with reindeer brucellosis [4-6, 9, 10, 12]. A study of cultural and biological properties of these strains revealed that they were completely identical to "deer" cultures (Br. suis biotype 4) [1-3, 17, 18]. This, of course, increases the probability of brucella infection in people who have no direct relation to deer-breeding, in such occupations as hunters, geologists, taiga highway builders and others. However, in spite of the significant number of reports dealing with studies of brucella cultures obtained from different species of animals inhabiting the Extreme North, some aspects of their biological characteristics have not yet been sufficiently investigated.

This work deals with a study of pathogenicity of cultures of brucella referable to the Br. suis biotype 4 species isolated from wolves, Arctic foxes, wolverines, deer and ermine. Reference culture Br. suis 40 served as the control. The cultures of brucella were kindly provided by Prov V. A. Zabrodin (Scientific Research Institute of Extreme North Agriculture, Noril'sk).

Material and Methods

Experiments were conducted on guinea pigs. The animals were infected hypodermically with a 2-day culture of brucella, in a dosage of 100 bacterial cells per mℓ. Autopsies were performed 30 days later for histological, serological and bacteriological studies.



Histological changes in guinea pig organs after infection with brucella cultures isolated from wild and game animals; magnification 260×

А) multiple small accumulations of epithelioid cells after infection with reference culture Br. suis 40

Б) extensive epithelioid granuloma with necrotic focus in regional lymph node after infection with brucella isolated from ermine

В) small necrotic focus in liver cells after infection with brucella from ermine

Г) large lymphoid foci, edema of alveolar septa in the lung after infection with brucella isolated from Arctic fox

We used several barrier organs for histological examination: spleen, liver, lung, as well as regional (inguinal) lymph nodes and lymph nodes far (cervical) from the site of injection of brucella. The material was fixed in 10% neutral formalin, imbedded in paraffin, and the sections were stained with hematoxylin-eosin.

Results and Discussion

Administration of reference strain Br. suis 40 to the animals induced significant pathomorphological changes only in the regional lymph nodes, in which numerous epithelioid proliferates appeared (see Figure), even in the presence of a generalized process. Irritation of follicles was observed in remote (cervical) lymph nodes and the spleen, indicative of intensified lymphopoiesis; in the liver there was a reaction referable to Kupffer's cells and in the lungs, edema of alveolar septa, multiple lymphoid foci and bands around the vessels, with occasional hemorrhages into the alveolar lumen.

Our study of histological material obtained from guinea pigs infected with cultures of brucella isolated from the Arctic fox, wolverine, ermine, deer and fox convinced us that these cultures varied in pathogenicity. Thus, the cultures of brucella isolated from the Arctic fox (Br. suis 280), ermine (Br. suis 221), wolf (Br. suis 99) and deer (Br. suis 171) were quite pathogenic to guinea pigs. Unlike the reference culture, Br. suis 40, when the animals were infected with the above-listed brucella cultures we observed development of epithelioid granuloma, not infrequently with necrotic foci, not only in regional (see Figure) but remoted lymph nodes, in relation to the site of injection of brucella. In addition, there was significant involvement of the liver, associated with appearance of nodules of histiocytic and reticular elements, small foci of necrosis and necrobiosis of hepatic cells (see Figure). There was also accumulation of exudate in the parenchyma, indicative of impaired permeability of vascular walls. The lungs presented the typical changes of brucellosis: lymphoid foci and bands around the vessels, edema of alveolar septa, occasional extravasations of blood into the alveolar lumen (see Figure).

The culture of brucella isolated from the wolverine (Br. suis 210) was considerably less pathogenic. Inoculation of this culture induced changes in organs and lymph nodes analogous to those observed in guinea pigs infected with reference strain Br. suis 40.

Our bacteriological and serological studies revealed that the highly pathogenic cultures usually induced a generalized process, and the antibody titer in the agglutination reaction was rather high (1:320) in such animals.

Conclusions

1. Cultures of brucella isoalted from the wolf, wolverine, deer, Arctic fox and ermine differed in pathogenicity to guinea pigs: those isolated from the wolf, Arctic fox, deer and ermine were highly pathogenic and induced severe

pathomorphological changes in organs and lymph nodes; the culture of brucella isolated from the wolverine was less pathogenic and was similar to reference strain Br. suis 40, with respect to morphological changes induced in organs and lymph nodes of the animals.

2. Deer cultures of brucella (Br. suis biotype 4), which circulate extensively, not only among wild and domestic reindeer, but migrate to many species of wild and game animals of the Extreme North and are highly pathogenic, may present some hazard to human health.

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UDC: 616.988.75-036.22-036.2-078.7

IMMUNOLOGICAL PRECURSORS OF INFLUENZA EPIDEMICS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5, 1978 pp 70-77

[Article by G. S. Skripchenko, Ye. M. Polyakov and N. I. Knyazeva, Odessa Scientific Research Institute of Virology and Epidemiology imeni Mechnikov, submitted 9 Mar 77]

[Text] It is extremely important to determine the actual start of an epidemic for prompt implementation of measures to control influenza. The theoretical premises and means of solving this problem were substantiated by data concerning the existence of subclinical forms of influenza in nonepidemic periods and similarity of immunological reactions of patients with clinically marked and subclinical forms of influenza. On the basis of these premises, Klyachko and Lamskaya [11] were among the first to describe intensification of circulation of influenza virus in interepidemic periods and established the existence of "latent outbreaks" of influenza against the background of no recorded elevation in morbidity rate. According to the data of Blaskovic [7], after latent circulation of the virus in an interepidemic period, antibodies to it can be demonstrated in 23% of those tested.

The latency period of an epidemic, which is actually the start thereof, may last up to 6 months. It can be detected only by means of a laboratory screening of the public [17, 19]. Determination of the start of this period and type of pathogen that induces a new epidemic would make it possible to gain sufficient time to implement specific preventive measures.

We investigated the immunological conditions under which epidemics are formed, the possibility of detecting their immunological precursors and some patterns of circulation and variability of influenza virus during periods preceding mass-scale rise in morbidity.

Material and Methods

We conducted our observations in stages: at first on a group from a machine-building enterprise (about 5000 people), then among the inhabitants of Odessa. Patients with acute respiratory diseases (ARD) who sought help in the medical and health unit of the plant, as well as individuals who had been in

contact with them on the job or elsewhere, were submitted to a random virological (isolation of virus on chick embryos) and serological (blood serum test with the reaction of inhibited hemagglutination (RIHA) screening by the conventional methods. In addition, we regularly tested blood serum of clinically healthy people in one of the plant shops in the course of 1962-1965. During 1967-1970, we screened polyclinic patients and volunteer blood donors. We compared the official records of morbidity to the results of the laboratory tests.

Results and Discussion

At the first stage of our study, the screening of individuals in the permanent group established that there was an increase in titer of antihemagglutinins, mainly to type B influenza virus, in the blood serum of essentially healthy people during the period preceding an epidemic of type B influenza (according to the records and laboratory tests on the sick, the epidemic developed in January 1963). Prior to the influenza A(H2N2) epidemic, which was recorded in February 1965, according to the morbidity records and laboratory diagnostics, and the pathogen of which was identified [16] as A2/Odessa/1/65, there was elevation of the titer of antihemagglutinins to influenza virus of the A(H2N2) variant in essentially healthy individuals. For the 3 months preceding the epidemic of influenza B, elevation of antibody titer to virus of this type was observed in healthy individuals 4 times more often ($P<0.05$) than to the virus of A(H2N2) influenza. On the eve of the A(H2N2) influenza epidemic the incidence of 4-fold or greater increase in titer to A(H2N2) virus was 8.45 times higher ($P<0.001$) than to type B influenza virus (Figure 1).

Figure 1.
Distinctions of serconversion to A(H2N2) influenza viruses (striped columns) and B virus (white columns) in essentially healthy members of the permanent group in different periods. X-axis, periods:
I) prior to influenza B epidemic
II) between epidemics of influenza B and A2
III) prior to epidemic of influenza A2

Y-axis, incidence of diagnostic seroconversion (%)

The increase in number of cases of influenza that were not diagnosed clinically in the period preceding the epidemic led to elevation of mean level of antihemagglutinins to the same subtype of virus, a subsequent variant of which then caused the epidemic. The increase in mean level of antihemagglutinins to type B virus and influenza A(H2N2) influenza virus in the group prior to the epidemics was attributable to a decrease in number of seronegative individuals and increase in number of people, in whom the antihemagglutinins were demonstrable in a titer of 1:40 or higher (Figure 2).

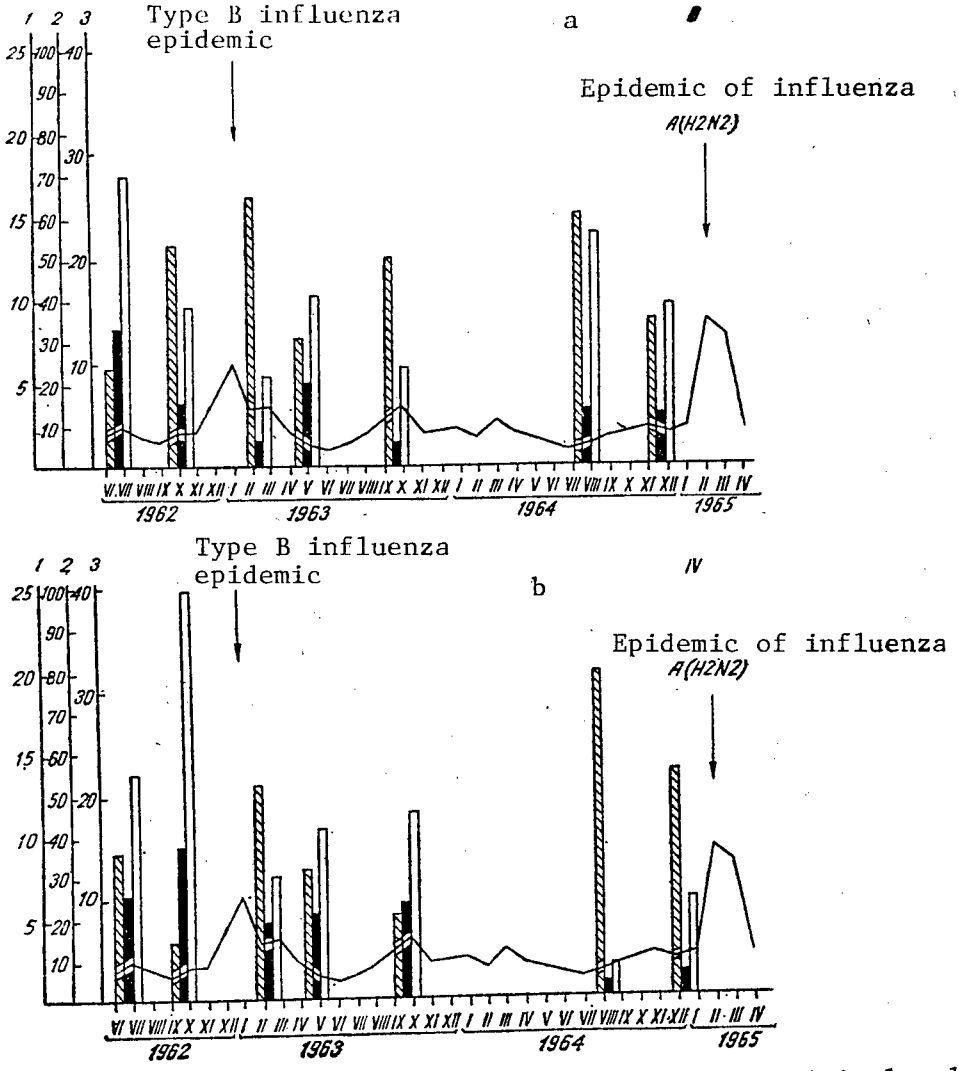

Figure 2. Incidence of influenza and antihemagglutinin levels in individuals of the continuously observed group [a--to influenza A(H2N2) virus and b--to type B influenza virus] Percentage of individuals with antihemagglutinin titer of less than 1:10 (columns with diagonal lines); percentage of individuals with hemagglutinin titer of 1:40 or higher (black columns); reciprocals of arithmetic mean antihemagglutinin titer (white columns). X-axis, observation periods; y-axis: 1) morbidity per 100 people
2) distribution of subjects according to antibody presence
3) reciprocals of arithmetic mean titer of antihemagglutinins

Such manifestations of increasing circulation of influenza virus were also observed in a sample of the urban population during the period of appearance of a "new" variant of the pathogen of influenza, serotype A(H3N2). In this case, the rise in antihemagglutinin level in blood serum to the precursor

virus was recorded 4 months prior to a marked rise in incidence of Hong Kong virus in January 1969. During the period of development of the second epidemic in this cycle, this time of an endogenous nature, considerably less time was required to overcome local confining factors. Immunoepidemiological manifestations of increased circulation of the virus were recorded 2 months prior to the rise in morbidity.

We were impressed by the fact that, after the first wave of Hong Kong influenza in 1969 and after the second, in 1970, the level of antihemagglutinins to precursor viruses of the Singapore variety was higher than immediately after circulation of these variants in 1967 during the epidemic that completed the 10-year cycle of Singapore influenza (1.90 and 2.61 times higher, respectively, with $P<0.001$). It should be noted that, after each elevation of antihemagglutinin level preceding the epidemics, the indices of collective immunity rose again at the start of the rise in morbidity (Figure 3).

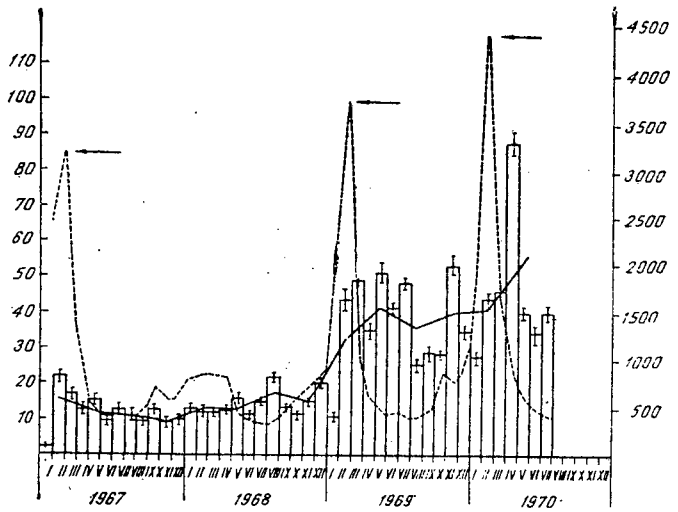

Figure 3. Dynamics of officially recorded incidence of influenza and ARS as compared to indices of humoral immunity to A(HN2) influenza virus in essentially healthy people of Odessa
Dotted line, mean daily morbidity in Odessa; solid line, mean quarterly level of antihemagglutinins among the residents; columns, mean monthly level of antihemagglutinins; line at the top of the columns, error of mean titer of antihemagglutinins; arrows, epidemics of H2N2 influenza. Y-axis, antihemagglutinin level on the left, incidence of influenza and ARS on the right.

Thus, regular screening of a sample of essentially healthy individuals indicated that one of the immunological precursors of influenza epidemics was an increase in number of cases of influenza in a subclinical form, detectable by laboratory tests, associated with elevation of titer of antibodies to the type of influenza virus a later variant of which induced the epidemic rise in morbidity.

Table 1. Immunological characteristics of influenza in clinically marked and subclinical cases, at different periods of development of epidemics

Influenza	Period	Cases of elevated antihemagglutinin titer	Mean antihemaggl. titer		Mean multiplicity of titer elevation	Reliability of differences between titers of 1st & 2d batches
			first batch of serum	second batch of serum		
B						
	Interepidemic (Jul--Sep 1962)	28	2,85±1,21	22,14±4,35	7,76	T=4,26 P<0,001
	Pre-epidemic (Oct--Dec 1962)	18	32,22±8,62	102,22±23,57	3,17	T=2,75 P<0,01
	Reliability of differences between means for periods:					
	T		3,37	3,34		
	P		<0,01	<0,01		
A2(H2N2)						
	Interepidemic (Mar 63--Nov 64)	23	9,13±2,45	26,95±4,71	2,95	T=3,32 P<0,01
	Pre-epidemic (Dec 64--Jan 65)	18	7,77±3,39	42,77±5,51	5,51	T=5,62 P<0,001
	Epidemic period (Feb 1965)	24	5,83±2,03	52,91±6,31	9,07	T=7,10 P<0,001
	Reliability of differ. between periods					
	T_{1-2}		0,32,	2,80		
	P		>0,1,	<0,01		
	T_{1-3}		1,03	3,16		
	P		>0,1,	<0,01		

The results of testing people who contracted a marked form of influenza in the nonepidemic periods prior to epidemics also indicated that formation of an epidemic population of the virus was associated with dominance thereof in the epidemic process. Thus, while the involvement of A(H2N2) and B viruses in the etiology of the sick cases was about the same ($P>0.1$) during the interepidemic periods (July--September 1962), on the even of the epidemic of A(H2N2) influenza there were 16 times more cases of marked influenza induced by a pathogen of this serotype than cases of influenza B ($P<0.001$). Prior to the influenza B epidemics, we succeeded in detecting only cases induced by the type B pathogen ($P<0.001$).

It was established that there are differences in conditions under which there was intensification of circulation of A(H2N2) and B influenza viruses. The initial antibody titer was about the same ($P>0.1$) in those who contracted influenza A throughout all periods of development of the epidemic. At the same time, the type B influenza virus acquired the capacity to induce the disease in the pre-epidemic period in individuals with stronger residual immunity, as compared to the interepidemic period. Thus, the initial level of antihemagglutinins in the serum of individuals who were sick prior to the epidemic was on the average 11.30 times higher than the initial level of those who were sick in the interepidemic period ($P<0.01$; Table 1).

The results of screening healthy individuals (see Figures 2 and 3), as well as those who contracted influenza (see Table 1), indicated that intensification of circulation of serotype A influenza viruses and morbidity occurred with a mean antihemagglutinin titer of about 1:10. Subsequent elevation of mean titers in essentially healthy individuals, to 1:20 or higher, preceded mass rises in morbidity. This warrants referring to arbitrarily "minimum" parameters of intensity of immunity, with which increased circulation of serotype A influenza viruses is possible, and "critical" parameters, on the basis of which one can judge the subsequent transition of the epidemic process into the phase of clinically demonstrable rise in morbidity. It may be assumed that these parameters are characterized by a different antihemagglutinin level in different climate and geographic regions, and that they differ from those established in Odessa.

We also observed an immunological distinction in manifestations of influenza during the process of development of epidemics, which consisted of a change in intensity of antibody production in those who had contracted influenza. In particular, among individuals who had suffered from influenza B, the titer of antihemagglutinins was 4.62 times higher throughout the pre-epidemic period than in those who had influenza in the interepidemic period. In the case of A(H2N2) influenza, the intensity of antibody production in response to infection in the pre-epidemic period was 1.59 times higher ($P<0.01$) and in the epidemic period 1.96 times higher ($P<0.01$) than in the interepidemic period (see Table 1).

There was a particularly graphic difference between frequency of seroconversion to A(H2N2) influenza virus in the inter- and pre-epidemic periods, as compared

to the epidemic periods in 1968-1969, with change in pandemic variant of the pathogen (Table 2). Thus, there was prevalence of 4-fold seroconversion (79.49%) in the inter- and pre-epidemic periods, with mainly 8-fold or greater seroconversion (61.40%; reliability of differences between periods, with regard to multiplicity of antibody titer elevation $P<0.001$) among individuals who contracted the disease. It may be assumed that immunoepidemic manifestations of this kind are a reflection of changes in immunogenicity of circulating viruses within the range of hypothetical forms of the main virus with "low level of activity" [3, 23, 25, 26] to "stages of the life cycle" with increased immunogenicity, the search for which is one of the most important tasks for medical science [31].

Table 2. Multiplicity of elevation of titer of antihemagglutinins to A(H2N2) influenza virus in cases of serological confirmation of the diagnosis in patients

Index	Period		Reliability of differences
	inter- & pre-epid. (May-Dec 68,69,70)	epidemic (Jan-Mar 69,70)	
Number of paired sera tested in RIHA	389	1438	
Frequency of diagnostic increment of antibodies: absolute	39	500	
%	12,02±1,52	34,77±1,25	
Distribution of diagnostic increment of antibody titer, multiplicity: 4			
abs.	31	193	
%	79,49±6,47	38,60±2,18	$T=5,99$, $P<0,001$
8: abs.	4	140	
%	10,25±4,85	28,00±2,01	
16: abs.	2	88	
%	5,12±3,53	17,60±1,70	
32: abs.	2	51	
%	5,12±3,53	10,20±1,95	
64: abs.	—	28	
%	—	5,60±1,03	
Overall, 8-fold or more: abs.	8	307	
%	20,51±6,47	61,40±2,18	For 8× increase or more; $T=5,99$, $P<0,001$

Thus, the results of surveying individuals who contracted ARS during the periods preceding influenza epidemics revealed that the following were precursors of the epidemics, in addition to those described above: progressive predominance of the pathogen of the developing epidemic in etiology of cases of influenza, rise of indices of group immunity from "minimum" to "critical" levels, increased multiplicity of seroconversion and antibody titer in those who became sick in the process of development of the epidemic.

The data pertaining to precursors of epidemics [15, 17-19] and research that confirms them [2, 6, 9, 10, 22, 24] are indicative of the existence of a latent stage of formation of epidemics of exogenous and endogenous origin. It may be assumed that during this period, which lasts 1.5 to 6 months, the pathogen undergoes ecological adaptation to local restraining factors. The latent stage of development of epidemics reflects the biphasic nature of rise in incidence of influenza. The first phase consists mainly of mild and clinical forms of the disease and the second, of clinically marked ones.

Probably, during the latent stage there is intensification of feedback between human defense capabilities and mechanisms of aggression of influenza virus. The heterogeneity of the population of the parasitic type increases as a result of increased activity of interacting systems. Evidently, residual immunity begins to play the role of a "sieve," while the adaptational mechanisms of the virus play that of a regulator of mesh size of this sieve.

Information, according to which predominance of "new" pandemic variants of serotype A influenza virus in 1957 and 1968 preceded intensification of circulation of the pathogens of past epidemics, can serve as an example of increased heterogeneity of the pathogen in pre-epidemic periods [1, 5, 7, 12, 13, 21]. In this regard, the data we obtained, indicating greater elevation of level of antihemagglutinins to H2N2 virus during the period of succession and dominance of variants of the new serotype (H3N2) can, to the same extent as analogous information of other researchers [6, 28, 30], be interpreted not only as the "natural" manifestation of antigenic heterogeneity of the population within the framework of the serotype, which precedes the predominance of a "new" pandemic variant of influenza virus, but also in other aspects.

It may be assumed that this phenomenon is based on mechanisms of "the original antigenic sin" [29]. On the basis of new data on the structure and properties of the population of influenza virus [4, 8, 14, 20], we cannot rule out either the probability of circulation of a pathogen with old, along with new antigenic groups during the epidemic period.

However, regardless of the mechanisms of the above-described phenomenon, the epidemic rise in incidence of Hong Kong (H3N2) influenza, in both 1969 and 1970, prior to initiation of immunization, was preceded by elevation of the titer of antihemagglutinins to the Precursor virus of the Singapore variety (H2N2). This warrants the conclusion that it is possible to detect immunological precursors of epidemics, not only in interepidemic, but interpandemic periods (during the succession of pandemic variants of influenza virus) using diagnostic preparations made form pathogens of prior epidemics.

Thus, the results of investigation of dynamic changes in population group immunity revealed that immunological precursors can be detected and a laboratory epidemiological forecast of influenza can be made by virologists in clinical laboratories through regular screening of population samples by means of conventional methods for the detection of influenza.

Conclusions

1. A seroepidemiological sampling of the population makes it possible to demonstrate precursors, reflecting intensified circulation of influenza virus, 1.5 to 6 months prior to a massive rise of morbidity induced by endogenous or exogenous viruses.

2. The following manifestations of increasing circulation of influenza virus at the latent stage of development of epidemics serve as immunological precursors: a) an increase in number of cases of subclinical form of influenza, associated with elevation of titer of antibodies to the type of influenza virus, the subsequent variant of which then induces an epidemic rise in morbidity; b) successive prevalence of the pathogen of a developing epidemic in the etiology of influenza; c) rise of indices of group immunity from "minimum" to "critical" levels; d) increased multiplicity of seroconversion and elevation of antibody level in those who contracted influenza in the course of development of the epidemic.

3. The most important immunoepidemiological characteristic of the novelty of the population of the pathogen of a developing epidemic is its capacity to stimulate in influenza victims a more marked seroconversion and accumulation of antibodies to a higher level at each successive stage of development of the epidemic, reflected by minimum immunogenic activity of viruses in the interepidemic period and maximum activity of viruses that circulate at the height of an epidemic.

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UDC: 616.988.73-036.22(479.24)

FINDINGS FROM A STUDY OF ORNITHOSIS INFECTION IN AZERBAYDZHAN SSR

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5, 1978 pp 83-86

[Article by F. A. Abushev, Azerbaydzhan Scientific Research Institute of Virology, Microbiology and Hygiene imeni Musabekov, Baku, submitted 16 Aug 77]

[Text] In the last few years, ornithosis infection has been acquiring increasing significance among infectious diseases of man. According to the data of Terskikh [30], over 130 species of birds referable to 19 orders and families and, apparently, their ectoparasites, are involved in the epizootic process. The results of studies made by Soviet [31, 32] and foreign [34, 35] authors revealed that endemic sites of ornithosis influence the formation, under certain conditions, of secondary or anthropurtic sites where human infection with ornithosis essentially occurs.

In view of the foregoing, as well as the favorable natural conditions and lack of data about ornithosis in Azerbaydzhan (with the exception of the work by Terskikh et al. [30], Sterkhova et al. [27]), we undertook a comprehensive study of this infection.

In 1966, we detected the first cases of ornithosis among patients with pathology of respiratory organs in Agdzhabedinskiy Rayon and among laboratory workers in Baku, who had examined rock-doves delivered from Agdzhabedinskiy Rayon [23]. As a result of our subsequent studies over a period of 8 years, we succeeded in detecting endemic and anthropurgic sites of this infection in various parts of our republic and to determine the immunological structure with regard to ornithosis, as well as to pick up cases of ornithosis among the inhabitants of different natural regions of the republic [1, 2, 9-11, 13, 25, 26].

Studies dealing with the detection of endemic sites of ornithosis were conducted in areas of maximum concentrations of birds (wintering and nesting sites) in the Kyzyl-Agach Sanctuary imeni Kirov, in the area of Lenkoran', on Glinyanyy Island in the Baku Archipelago and in the lakes of the Sary-Su system.

Over a period of 2 years (1967-1968), serological studies (CFR [complement fixation reaction] and NCFR [neutralization of CFR?]) were made at the Kyzyl-Agach Sanctuary of 494 birds referable to 10 speices; specific antibodies to the pathogen of ornithosis were demonstrated, in titers of 1:10-1:40, in the blood serum of 65 of them (13.15%). Of the 10 species of birds surveyed, 5 were seropositive for the pathogen of ornithosis (heron--N. nyctocorax L., pygmy cormorant--Ph. pygmaeus Pall., little egret--E. garzetta L., squacco heron--A. ralloides S., starling--S. vulgaris L.), and we first demonstrated seropositive reactions in the squacco heron and heron in 1967 [9-12].

The results of the studies revealed that an endemic site of ornithosis, demonstrated in 1954-1955, retained activity 15 years later.

Over a 2-year period (1970-1971), we surveyed on the Sary-Su lakes and surrounding territories a total of 141 birds of 29 species, referable to 9 orders; antibodies to the pathogen of ornithosis were demonstrated in 40 of them (28.37%). Four species of the order Ciconiiformes (purple heron--A. purpurea L., squacco heron, little egret, spoonbill--P. leucorodia L.), three species of the order Passeriformes (jackdaw--C. monedula L., starling, shrike--L. minor L.), two species of the order Charadriiformes (thickfoot--Oedicnemus L., black-winged stilt--H. himantopus L.), one species of the order Coraciae (roller--C. garrulus L.) and one from the order Columbiformes (rock-dove--Columbia livia L.) were seropositive. Among these species, infection of purple herons, spoonbills, starlings and black-winged stilts was demonstrated for the first time [5].

The endemic sites of ornithosis demonstrated in the Kyzyl-Agach Sanctuary and Sary-Su lakes can be classified as the polyhost type, since 11-12 bird species are involved in circulation of its pathogen.

Over a period of 5 years (1968-1971, 1973) we screened on Glinyanyy Island a total of 532 herring-gulls and 36 terns and found that the mean incidence of infection constituted 13.09% among the gulls (L. argentatus L.). We succeeded in isolating the pathogen of ornithosis in one out of 89 examined gulls. Evidently, O. capensis fowl ticks play some role in the epizootic process, since they strike fledgling gulls on a mass scale (index of profusion 4.0, and extensiveness of infection 25.2), and they are capable of retaining the pathogen of ornithosis, after experimental infection, for 6 months and of transmitting it to sensitive birds (chicks) [15-17]. According to its structure, the endemic site of ornithosis on Glinyanyy Island should be classified as the dihostal type, since two species of birds are involved in the epizootic process: herring-gull and common tern (S. hirundo L.).

The endemic sites of ornithosis on Glinyanyy Island, Sary-Su lakes and in the Kyzyl-Agach Sanctuary are mixed, since in these territories endemic sites of ornithosis, arboviruses (Tahyna, Uukuniemi, Sindbis, West Nile, Sumakh, Baku), rickettsiosis (tickborne Asian typhus, Q fever) and leptospirosis [11-15, 17-25, 27-31, 33] have been demonstrated.

In view of the lack of data pertaining to anthropurgic sites of ornithosis in Azerbaydzhan and the suspected possibility of existence thereof, in 1966 to 1973, we examined rock-doves in 13 regions, pheasants in one, domestic fowl in 9 regions and cities situated in various natural areas of the republic.

A total of 2095 rock-doves were examined in 13 populated centers of natural regions of Greater Caucasus, Lesser Caucasus and the Kura-Arak plain; 693 of them (33.06%) presented specific antibodies to the pathogen of ornithosis in titers of 1:10-1:160. The amount of positive findings ranged from 8.82% to 52.3%. In two centers (village of Agdzhabeda, Baku), pigeons were submitted to biological testing, and this enabled us to isolate the pathogen of ornithosis [3, 5, 23].

Tests on 1741 domestic fowl (chickens, turkeys, ducks, geese) in 12 poultry farms revealed 17.0% seropositive reactions. The number of fowl (chickens) with antibodies to ornithosis ranged from 9.86 to 43.33 in different years and different farms [7, 8, 14].

Biological and allergological tests demonstrated that pheasants were infected with ornithosis at the Abdulyanskiy Pheasant Farm in Sabirabadskiy Rayon [7].

In view of the presence of endemic and anthorpurgic sites of ornithosis in Azerbaydzhan, we performed serological tests on blood serum of different population groups over an 8-year period (1966-1973), for demonstration of the pathogen of ornithosis. In all, we tested serum from 14,479 sick individuals suffering from various diseases, and positive results were obtained in 1459 (10.08%) [2, 6, 9, 12, 23, 26].

The results of the study indicate that the public of the republic is infected (according to serological data) the year round, while more intensive infection is observed in some natural areas and regions in the autumn-winter and spring.

Our data do not indicate that there are differences between incidence of ornithosis in males and females.

The highest incidence of seropositive reactions among the above patients was found in the age group of 41-60 years (11.81%).

Antibodies to the pathogen of ornithosis were more often demonstrable among patients with pathology of respiratory organs, which is consistent with the findings of other researchers, who found that the lungs were involved in 10-25.5% of the ornithosis cases [30].

A serological screening of 321 individuals who were in constant contact with birds revealed that 20.25% had antibodies to the pathogen of ornithosis. In another group of such individuals (407 people), a positive reaction to intracutaneous injection of allergen was found in 40.29% of the cases [14]. A comprehensive survey of this population group made it possible to retrospectively diagnose 38 sporadic cases of ornithosis.

Between 1966 and 1973, 155 cases of ornithosis were found in all physico-geographic regions of Azerbaydzhan [3, 4]. Of this number, 58 were picked upon in the course of a complex survey of 316 patients, among whom 25% (79) presented a positive CFR and 38.28% (21%), positive allergic test. The main source of infection of these patients was referable to domestic (chickens, ducks, turkeys), ornamental (Australian love bird--M. undulatus L., pigeons--C. livia L., and others) and synanthropic (rock-doves and others) birds. It must be stressed that mainly sporadic cases of ornithosis are observed in Azerbaydzhan.

The above data indicate that ornithosis is one of the widespread diseases in Azerbaydzhan, and it is found in all of its natural regions. This is attributable to the presence of active endemic and anthropurgic sites of this infection in the republic, for the prolonged existence of which the natural conditions are favorable.

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UDC: 616.981.455-036.21-07(470.324)

SOME PATTERNS OF CHANGE IN NUMBER OF CARRIERS AND EPIZOOTIC ACTIVITY IN A LOCAL ENDEMIC SWAMP SITE OF TULAREMIA IN VORONEZHSKAYA OBLAST

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5, 1978 pp 133-138

[Article* by V. R. Krasil'nikov and V. S. Sil'chenko, Oblast Sanitary and Epidemiological Station, Voronezh, submitted 27 Jul 77]

[Text] The Povorino endemic tularemia site has long since attracted the attention of researchers [7, 8, 12-14], as a classical example of a local swampy site. It is situated in the northeastern part of the oblast, in the floodplain of the Khoper and Svintsovka rivers. It is over 6000 ha in size. The location of this site on the boundary between forest-steppe and steppe regions is the reason for a significant abundance and diversity of fauna [11, 12]. There are over 35 species of rodents, insectivores and carnivorous mammals inhabiting this region. The most frequently encountered animals are the water and common rebacked vole, common field, striped field and house mouse. The yellow-necked field mouse, nutria, common shrew, water shrew and desman [or muskrat] are commonly found in these areas. Blood-sucking insects are widely represented by horseflies and clegs, mosquitoes, buffalo gnats and biting midges. Ixodes ticks are represented by Ixodes ricinus and Dermacentor marginatus, but they are few in number.

Epizootic and epidemiological observations of the site have been pursued since 1930,** but they became organized and planned in 1958. In the period from 1958 to 1974, a total of 19,326 mammals (Table 1), 98,700 blood-sucking Diptera, 408 ixodes ticks and 1672 samples of water were examined. As a result, 370 cultures of F. tularensis were isolated, including 352 from the water vole, 4 from the striped field mouse, 1 from a water shrew and 13 from water. No cultures were isolated from ticks.

The study of dynamics of number of rodents and epizootic activity between 1958 and 1974 enabled us to divide this period into two parts. The first (1958-1968)

*Dedicated to S. V. Suvorov, A. A. Vol'ferts and M. M. Voronkova, who were the first to discover tularemia in the USSR.

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was characterized by a large population of water voles (35-88% trapped along the shoreline during the spring flood tides), while the number of other rodents held at a low level druing this period (maximum of 16.5% trapped). The second period (1969-1973) began with an exceptionally rigorous, snowless winter, which induced, along with drying of marshes, a profound depression in the population of water voles, which continued to the end of this period; the number of small rodents was rapidly restored and, by the summer of 1970, reached a high level (50-70% trapped).

Table 1. Species composition and number of animals tested

Animal species	Number of animals		Number of isolated cultures	
	abs.	%	abs.	%
Water vole	17 654	91,35	352	98,6
Common redbacked vole	574	2,97	—	—
Common vole	67	0,35	—	—
Striped field mouse	707	3,66	4	1,1
Common field mouse	132	0,68	—	—
Yellow-necked "	44	0,23	—	—
House mouse	52	0,26	—	—
Common shrew	73	0,38	—	—
Water shrew	7	0,04	1	0,3
Other mammal. species	16	0,08	—	—
Totals	19 326	100,0	357	100,0

It was established that all epizootic outbreaks in the site were referable to the period of a large population of water voles, i.e., before the winter of 1968-1969. There was extremely negligible involvement of other rodents in the tularemia outbreak, 1.4% of the total number of cultures isolated. After 1969, we failed to demonstrate epizootic tularemia, in the presence of a large population of small rodents (Figure 1).

The results of subsequent investigation of epizootic activity of the Povorino endemic swampy site of tularemia warranted considering it monohostal [10]. Epizootic outbreaks were observed primarily among water voles, and they determined the epizootic adversity of this site [4].

Evidently, a minimum (threshold) population of water voles, which are the main carriers of the pathogen of tularemia infection, is required for the epizootic outbreaks to occur. In the endemic Povorino site, this level was about 30% of the trapped water voles. It may be assumed that this is close to the threshold level.

Between 1958 and 1968, when the water vole population was always above the threshold level, there were virtually yearly epizootic outbreaks of tularemia. However, their intensity varied, and it was unrelated to fluctuations in number of carriers. Thus, the 1958 outbreak occurred at a 80% trapped level,

while in the spring of 1959, when there were somewhat more water voles, no outbreaks occurred, although more animals had been tested than in 1958 (Table 2). By the spring of 1960, the trapped level dropped to 56% and, in spite of this, there was an epizootic outbreak of tularemia (61 cultures of F. tularensis per 1000 tested animals). In 1961, water voles multiplied intensively (52% trapped), but there were no epizootic outbreaks.

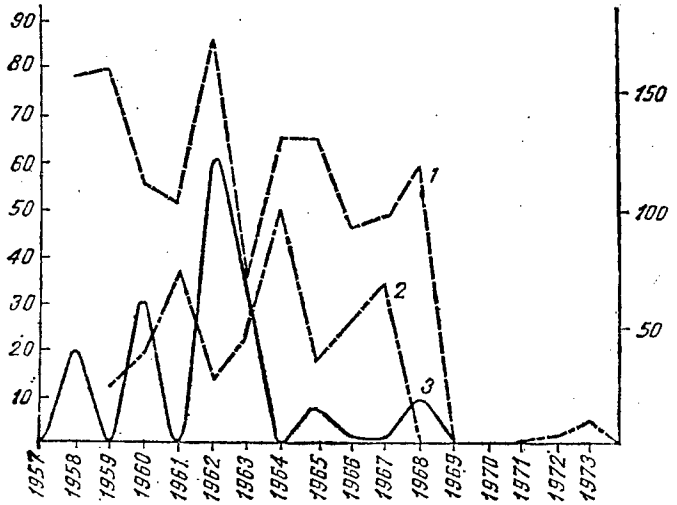

Figure 1.
Dynamics of water vole population size and intensity of epizootic outbreaks of tularemia in the Povorino endemic site.
Y-axis, % trapped on the left; number of cultures isolated per 1000 tested animals on the right

1) population of water voles in the spring (flood tides)
2) in the fall
3) intensity of epizootic outbreaks

Table 2. Number of animals examined in different years

Year	Water vole			Other species			Total animals tested	Cultures isolated fm water	Number of cultures	Cultures/ 1000 tested
	number of tests	% trapped	number of cultures	number of tests	% trapped	number of cultures				
1958	1 270	80,0	4	23	—	—	1 293	—	4	3,1
1959	1 997	80,5	—	110	16,5	—	2 107	—	—	—
1960	1 030	56,7	67	71	10,4	—	1 101	—	67	61
1961	1 262	51,7	—	34	10,3	—	1 296	—	—	—
1962	1 184	88,5	167	185	15,7	2	1 369	2	171	123
1963	1 011	35,3	52	68	18,5	—	1 079	4	56	48
1964	1 005	66,6	—	16	5,5	—	1 021	—	—	—
1965	2 172	65,5	31	47	9,3	—	2 219	4	35	14
1966	2 358	46,3	4	229	10,3	1	2 587	—	5	1,9
1967	2 868	49,1	1	156	11,7	2	3 024	—	3	0,9
1968	1 478	60,1	26	51	9,7	—	1 529	3	29	17
1969	—	—	—	42	7,8	—	42	—	—	—
1970	1	0,5	—	298	49,2	—	299	—	—	—
1971	—	—	—	146	50	—	146	—	—	—
1972	—	—	—	14	16	—	14	—	—	—
1973	16	2,0	—	83	216	—	99	—	—	—
1974	2	2,0	—	99	22,5	—	101	—	—	—
Totals	17 654	—	352	1672	—	5	19 326	13	370	—

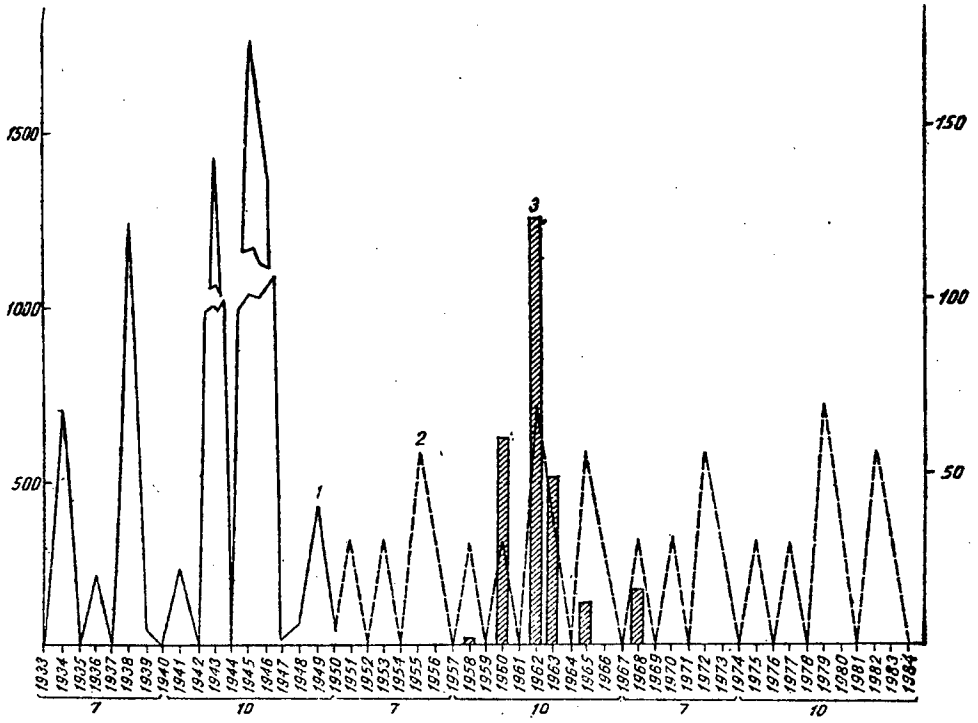

Figure 2. Incidence of tularemia in Povorinskiy Rayon and intensity of epizootic outbreaks. X-axis, years; y-axis, number of cases per 100,000 population, on the left, and number of cultures isolated per 1000 tested animals, on the right.

1) number of sick cases per 100,000 population
2) hypothetical morbidity curve
3) number of cultures isolated per 1000 tested animals

In 1962, we found the largest population of water voles over the entire observation period, and among them the most intensive epizootic outbreak was demonstrated (123 cultures per 1000 tested animals). It continued in 1963, but did not affect the size of the water vole population, which multiplied intensively and, by the spring of 1964, the trapped level constituted 66%, but there was no epizootic outbreak that year. In 1965-1967, the dynamics of intensity of epizootic outbreaks were identical to the dynamics in 1962-1964, but they were not as acute.

Thus, there was a sharp change in intensity of epizootic outbreaks between 1958 and 1968. It increased from 1958 to 1962 and decreased by 1968. The elevations and drops were not uniform, but undulant, at 2-3 year intervals. The phase of growth in intensity consisted of 2 waves at a 2-year interval (1958-1959, 1960-1961). The phase of decline consisted of 2-3 summer waves (1962-1964, 1965-1967). The entire cycle took 10 years.

Long-term observations of size of water vole population led to the conclusion that the epizootic outbreaks of tularemia in the Povorino site definitely influenced it, but this influence was not always the same, and it depended on the phase (rise, decline) of epizootic activity. At the phase of elevation, when the outbreaks had a 2-year cycle, the number of water voles dropped significantly (1958, 1960); at the phase of decline in intensity of the outbreaks, which occurred in the form of 3-year waves, there was a sharp decrease in number of water voles the first year (1962, 1965), whereas in the second year, in spite of the continuing epizootic situation, their number was restored (1963, 1966).

Table 3. Incidence of tularemia among people and isolation of F. tularensis in the Povorino site

Year	Number of cases	Number of cultures	Year	Number of cases	Number of cultures isolated
1930	2	—	1951	4	1
1934	103	—	1952	3	2
1936	32	—	1953	1	—
1938	183	37	1958	1	3
1939	6	2	1959	—	4
1941	33	—	1960	1	67
1943	377	—	1962	2	171
1945	1481	3	1963	1	56
1946	347	3	1965	—	35
1947	1	—	1966	—	5
1948	10	6	1967	—	3
1949	62	—	1968	—	29
1950	5	—	1969—1974	—	—

Notes: 1. In the years that are not listed here, there were no cases of tularemia among humans and no cultures were isolated.

2. In 1943 and 1945-1946, the cases of tularemia among humans were related to epizootic outbreaks among mouse-like rodents, common voles and house mice.

The epizootic outbreaks of tularemia, which caused significant fluctuations in number of water voles in different years [14], could not have induced a lasting decline thereof. Abiotic factors and man's activities were the cause of a persistent decline in population size [5, 6, 9]. When the number of water voles dropped below the threshold level, the site moved into an epizootically inactive phase. Kalabukhov [2], Karpov [3] and Kondrashkin [4] arrived at the same conclusion with regard to flood plain sites.

To provide a fuller description of the changes in epizootic activity of the Povorino site, we analyzed data referable to the incidence of tularemia among people who were in the region of this site (water vole trappers, water consumers, agricultural workers, etc.). The results obtained (Table 3) revealed

that the morbidity rate varied significantly in different years (Figure 2); years that were uneventful, with regard to tularemia, were follwed by years of a significant incidence of tularemia among humans. We were impressed by the coincidence of succession of periods of epizootic activity and epidemic uneventfulness. Here too, we observed rises and declines of infections, the same cycles and waves as in the epizootic outbreaks. Between 1933 and 1939, we recorded one 3-year and two 2-year waves of morbidity, the entire cycle lasting 7 years. The 10-year cycle from 1940 to 1949 consisted of two 2-year waves. From 1946 on there were significant changes: a sharp decline in outbreaks of tularemia followed by a transition to isolated, sporadic cases. From this year on, planned preventive inoculations against tularemia began to be administered on a regular basis.

If we assume that there are alternate 7- and 10-year cycle and were to plot the hypothetical curve of morbidity on this basis (which is also the curve for epizootic breaks), from 1958 on we would find that it coincided completely with the actual curve of epizootic activity. After 1974, the population of water voles began to grow, in 1975 and 1977 epizootic outbreaks of tularemia were found among them, which corresponded to our projected curve of epizootic activity. Investigation and demonstration of the distinctions of dynamics of epizootic tularemia, the effects thereof on the number of voles, which are carriers of this infection, make it possible to formulate a long-term forecast and plan epidemic-control measures in endemic sites of tularemia in flood plain and swampy areas.

Conclusions

1. In a swampy endemic site in Povorino, epizootic outbreaks of tularemia occurred when there was a large number of water voles; other rodents and ixodes ticks did not play an appreciable role in maintaining the endemicity of the infection.

2. The threshold number of water voles, which presented a threat of epizootic outbreaks of tularemia, constituted 30% trapping rate; when the number thereof was above the threshold, the fluctuations in epizootic activity of the site were unrelated to changes in number of carriers.

3. In this site, epizootic activity was characterized by alternation of 7- and 10-year cycles, including a certain number of waves occurring in 2-3-year periods; during the phase of growth in intensity of epizootic activity (2-year waves) there was a significant decrease in number of water voles, during the phase of decline (3-year waves) their number dropped sharply in the first year and rose in the second, in spite of the epizootic activity.

4. Cases of tularemia among humans in the region of the site usually coincided with periods of high epizootic activity of water voles.

5. The demonstrated distinctions in the dynamics of epizootic activity and its influence on number of the main carriers of tularemia infection made it possible to formulate a long-term forecast and plan epidemic-control measures.

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INTERNATIONAL RECOGNITION OF SOVIET PUBLIC HEALTH

Moscow ZDOROV'YE in Russian No 6, 1978 pp 2-3

[Article by Academician B. V. Petrovskiy, USSR Minister of Health]

[Text] Our entire life and constructive creativity are still glowing from the recent festivities celebrating the glorious 60th anniversary of the Great October Socialist Revolution, which became a historical landmark in the fates of the nations of our country and the entire world. Great October originated the revolutionary transformation of the world, heralding the coming of a new, communistic era in development of mankind.

This noteworthy anniversary was celebrated, along with the entire country, by Soviet public health, the offspring of Great October.

At all of the stages of its heroic route, the Communist Party devoted enormous attention to safeguarding the health of the working people. Already in the first program, prepared by V. I. Lenin and adopted in 1903, the Party demanded establishment of an 8-hour work day, free medical care, social insurance and sanitary surveillance at enterprises "in the interests of protecting the working class from physical and moral degeneration, as well as in the interests of development of its capacity for fighting for liberation."

The party of communists arrived at the October assault of the old world with Lenin's ingenious program for socialistic transformation of society, a strategic plan for development and strengthening of the new state, its economy, science and culture. Concern for the health and welfare of the people was an integral, organic part of this plan.

On 11 July 1918, V. I. Lenin signed the Edict pertaining to establishment of the People's Commissariat of Health. From that day on, public health care became an integral part of the national policy of the world's first socialist state, which took on the concern for safeguarding and constantly improving the health status of all the people.

In the 60 years of socialist public health care, Soviet medicine has made great advances in all areas of scientific, clinical and preventive medicine.

The material, technical and organizational conditions were provided so that all of the citizens of our country could receive free, accessible and qualified medical care.

The achievements and knowhow of our state, which resolved comprehensively the problem of public health care and guaranteed, in its constitution, this right to each Soviet citizen, are of enormous international significance, constituting a lofty example of social justice and humaneness to millions of people all over our planet. This knowhow is studied, not only by our friends in many countries of the world, but our ideological opponents.

The national nature of Soviet health care, its large scale and complexity, planning and scientific substantiation, its preventive direction and availability to all, profound democracy and genuine humaneness have become an extremely great magnetic force.

The international prestige of Soviet medicine has grown to an unprecedented extent. Our country is implementing broad collaboration with fraternal socialist countries; it shares generously its achievements and knowhow in the area of public health, as well as renders selfless assistance in organizing medical care for the people of developing countries. The USSR plays a large role in the activities of the World Health Organization; we are also collaborating fruitfully in the area of medical science and organization of public health care with a number of developed capitalistic countries.

In their multifaceted activities on the international area, Soviet medical scientists and public health organizers are governed by the decisions of the 24th and 25th CPSU congresses, as well as the Program for Peace, in which there is expression of readiness "... to deepen mutually beneficial collaboration in all areas with nations that, in turn, strive toward the same goal, ... to participate with other concerned nations in solving problems such as preservation of the environment, harnessing energy and other natural resources, developing transportation and communications, preventing and eradicating the most dangerous and widespread diseases, exploring and conquering space and the world oceans."

The exchange of ideas and opinions in the field of medical science may expedite solutions to such extremely difficult problems as determination of the causes of malignant neoplasms, development of methods of prevention and treatment of oncological, cardiovascular, neuropsychiatric and other diseases. Collaboration in the most humane area of knowledge, businesslike contacts between scientists from different countries will, no doubt, aid to a considerable extent in international detente.

The Soviet Union, on the example of its knowhow, has revealed to the entire world the close correlation between public health care and the socioeconomic development of a nation.

The great Lenin repeatedly stressed that the health of each member of society is a national asset, "government property." These principles and humanistic

ideas of Lenin, of paramount importance, are being consistently implemented by the entire set of national measures for continued improvement of public health care. These principles are the universal key as well for the solution of one of the most important social problems of many nations, who are facing a choice with regard to direction of their sociopolitical development.

In the last 60 years, Soviet public health has demonstrated thoroughly the decisive importance of the preventive direction of medicine, which is based on complex implementation of social and sanitary-hygienic tasks. Our entire Soviet legislation dealing with public health, Party and government decrees are permeated with this principle, being directed toward amelioration of the environment, living, working and recreational conditions for the workers, creation of beneficial conditions for development of the creativity of the individual.

The principles of prevention are of extreme importance to the matter of eradicating particularly dangerous infectious diseases. The Soviet Union is collaborating with many countries, within the framework of WHO, as well as on the basis of bilateral agreements in the struggle to prevent and eradicate the most dangerous and widespread diseases, and environmental protection. Implementation of the program for eradication of smallpox from the entire world, participation in measures to protect the oceans from pollution by industrial waste are examples of this.

The selfless work of Soviet medical specialists in developing countries of Asia, Africa and other regions, which have freed themselves from colonialistic bondage, is also gaining international recognition. The fruitful work of Soviet physicians in Algeria, Guinea, Mali, Chad, Nigeria, Congo, Zambia, Mozambique, Angola, the People's Democratic Republic of Yemen, Afghanistan and Ethiopia has demonstrated to the people of these countries the humaneness and high professionalism of emissaries from the country of victory of socialism. Comrade L. I. Brezhnev, general secretary of the Central Committee CPSU, speaking from the rostrum of the 25th CPSU Congress, expressed his sincere appreciation to Soviet specialists working abroad, including physicians, "for their great understanding and conscientious performance of their international duty."

The training of national medical cadres at Soviet VUZ's is of inestimable aid to developing countries. The land of the Great October Revolution has opened wide the doors into the temple of science to emissaries from a young generation of nations struggling for freedom and independence, defending their sovereignty against the intrigues of neocolonialists. Over 4000 physicians, as well as hundreds of feldshers and nurses have been trained for these nations at Soviet VUZ's and medical schools. At the present time, more than 5000 students and residents from 95 countries of the world are studying in the USSR.

The facts speak for themselves with respect to the importance and prestige of Soviet medical science and public health. Soviet scientists participate actively in the work of many international, intergovernment organizations, such as the International Agency for Cancer Research, UNICEF, WHO, 40 international

scientific societies and associations, international and national medical congresses and conferences abroad; they receive numerous delegations of prominent medical scientists and practicing physicians from many countries of the world. The largest biomedical and organizational problems, important to all of mankind, are being solved with the participation of Soviet specialists.

Our country has an enormous scientific medical potential: every fourth physician in the world is a Soviet citizen! It is also directed toward comprehensive analysis and exploration of the means of solving national, regional and worldwide problems of public health that require the joint efforts of many countries. The Soviet Union is comprehensively involved in coordinating scientific research programs dealing with the control of such widespread diseases as cancer, cardiovascular, endocrine, metabolic, infectious, viral, tropical and parasitic diseases.

Organization of the public health service in fraternal socialist countries is based on the principles of Soviet public health care. There is a special commission under CEMA, as well as annually convoked meetings of ministers of health of socialist countries, to coordinate multilevel collaboration. In 1976, a program of great theoretical importance was approved at such a meeting: "Main directions and prospects of development of socialistic public health," which then became the official document of the 30th World Health Assembly.

A special resolution of the World Health Assembly, "On the main principles of development of national health care," will also have a strong influence on development of national health services. It is based on socialistic principles, which have been recognized as "the most effective and tested in a number of countries." The worldwide medical community will have another opportunity to appreciate the achievements and knowhow of socialist public health at an international conference of WHO dealing with development of primary medical and health care, which will convene in Alma-Ata in October of this year.

With each year, the prestige of Soviet health care on the international area is growing and strengthening. The example of the country of developed socialism, in which concern for public health has been promoted to the rank of a social problem of paramount importance, is exerting an increasing influence on the ideas and feelings of millions of people, bringing to the people of the world the truth about the most just society on earth, about the future of mankind.

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CSO: 1870

TOBACCO SMOKE AND VITAMIN C

Moscow ZDOROV'YE in Russian No 6, 1978 p 32

[Article by Prof N. G. Bogdanov]

[Text] It is a known fact that when tobacco burns there is production of significant quantities of diverse substances, many of which are not indifferent and even harmful to human health. We refer to nicotine, ammonia, hydrocyanic acid, carbon monoxide and others, including those that cause malignant degeneration of cells.

First of all, smoking has a deleterious effect on respiratory organs. Smokers often suffer from various chronic diseases of the pharynx, larynx and bronchi. They are more susceptible than nonsmokers to the danger of cancer of the tongue, lower lip, larynx and lungs, as well as peptic ulcers, serious diseases of the heart and lungs. It has been proven that by inhaling tobacco smoke man dooms himself to hypoxia. The fact of the matter is that part of the hemoglobin is stably bound with carbon monoxide, forming oxyhemoglobin, which is not capable of delivering oxygen to cells and tissues.

Research of recent years has supplied new facts indicative of the devastating effect of tobacco smoke on the body. It was found that smokers usually have a vitamin C (ascorbic acid) deficiency.

There were two groups of men under observation by researchers, smokers and nonsmokers, who were given the same amount of ascorbic acid with their food. This is what laboratory tests showed: the vitamin C level in blood plasma and leukocytes of smokers was always 50% lower than in nonsmokers. The same pattern was established in a study of smoking and nonsmoking women.

Numerous investigations indicate that the vitamin C deficiency in smokers is due to poor assimilation of ascorbic acid.

Both ascorbic acid and its oxidized form, dehydroascorbic acid, have equal vitamin C activity. Vitamin C usually passes through the cell membrane of the small intestine in the form of dehydroascorbic acid, which is then readily converted into ascorbic acid. It was found that the process of conversion of

dehydroascorbic acid into ascorbic acid is sharply depressed in smokers. For this reason, even with adequate intake of vitamin C, the body experiences a deficiency.

Obviously, researchers were concerned with the question of which of the numerous substances formed when tobacco burns is the most guilty in disrupting the vitamin C balance in the organism.

Acrolein was found to be the main culprit; it is one of the components of tobacco smoke. Expressly this substance, which penetrates readily in the body, prevents the conversion of dehydroascorbic acid into ascorbic acid. Even when it penetrates in the body in small quantities (when an individual smokes little), acrolein is active for a long time and performs its dark deed. For this reason, C hypovitaminosis is observed not only in avid smokers, but those who smoke less than 15 cigarettes a day. Furthermore, a vitamin C deficiency also develops in passive smokers; i.e., those who do not smoke but are exposed to tobacco smoke daily.

It is known that vitamin C is actively involved in vital functions. It is involved in transformation of one vitamin, folic acid, into its active form, which participates in hemopoietic processes. Vitamin C also plays a large role in assimilation and deposition of iron in the body, in synthesis and metabolism of adrenal and thyroid hormones, which are the most important regulators of metabolic processes. Ascorbic acid provides for a high level of immunological reactions; it is involved in detoxification of some chemicals in cases of poisoning and it performs numerous other important functions in the body. The smoker, who deprives himself of vitamin C, causes much detriment to his health.

Irritability, fatigability, poor appetite, sleep disorders and frequent colds are attributed by the smoker to any causes, but not the real one, C hypovitaminosis induced by smoking.

The ascorbic acid deficiency has a particularly deleterious effect on the health of children. For it is during the period of growth and formation that the body is very sensitive to a shortage of vitamins, incluidng vitamin C. Yet this deficiency is rather significant in children and adolescents who either adopted from adults the devastating smoking habit, or were compelled to breathe air saturated with tobacco smoke. It is imperative to give up smoking. It is imperative in the interests of your own health, as well as in the interests of the health of your children!

10,657
CSO: 1870

PROTEIN USED AS PROTECTION AGAINST VIRUSES

Moscow ZDOROV'YE in Russian No 6, 1978 pp 6-7

[Article by V. D. Solov'yev]

[Text] Timely problems of modern medicine are discussed regularly at meetings of the Presidium of the USSR Academy of Medical Sciences. One of them is the "Interferon" program. Academician V. D. Solov'yev, of the USSR Academy of Medical Sciences, tells our correspondent, T. V. Mashkevich, about the directions in which work is being pursued to develop methods of producing interferon and decoding its properties.

Twenty years have passed since interferon became known to science. It was discovered by virologists, the Englishman Isaacs and Swiss national, Lindeman, who are on the staff of the London National Institute. This discovery, like most similar ones, was made by chance during experimentation. The researchers were trying to determine why, after viruses are injected in a cell, some of them prevent reproduction of others. The investigators assumed that the viruses do not engage in "hand to hand" battle with one another, but use a supplementary means, protein. The same scientists then discovered it. And since the antagonism of viruses was originally called interference, the discovered low molecular protein was named interferon.

At the present time, this protein is one of the recognized factors of immunity, immunological homeostasis and protection of stability of the body's internal environment. Unlike its "comrades in arms," antibodies, interferon acts only within the cell and does not travel beyond its boundaries. Antibodies, which are formed in lymphoid tissue and migrate into blood, fight against extracellular enemies. They are always specific and interact only with the viruses, in response to whose invasion these antibodies were formed. Interferon, however, protects the cell from virtually all viruses, so that it may be considered a factor of general, nonspecific protection of the organism. There is another distinction: The antibody deprives a virus of aggressiveness, uniting with it "unto death," while interferon does not unite with virus, but strikes from within, destroying the genetic mechanism of its reproduction.

The process of production of interferon is extremely complex, and it has not been completely identified. Thus, it is still not known whether the low molecular protein is present in the cell before a virus penetrates into it. We can only state with certainty that the amount of interferon begins to increase immediately after the virus has invaded the cell.

Research also revealed some other important facts. In particular, it was learned that interferon is produced more slowly and in smaller amounts in infants and elderly people (over 65 years old) than in representatives of other age groups. This protein is also produced less intensively in cold weather. All this explains why infants and the aged are more seriously affected by respiratory viral diseases, as well as why these infectious diseases strike more often in the late fall and winter. It may be assumed that physical training and conditioning, which activate metabolism in cells, including interferon production, strengthens the defense strongholds of the body, its resistance to deleterious environmental factors.

In the very first years of investigation of interferon, it was proven that it is formed both in the body and in vitro, in isolated cells in cultures. This made it possible to undertake the production of interferon and build up a large supply thereof, which permitted investigation of its therapeutic and preventive action. Interferon as a therapeutic agent is valuable because it is harmless to the body and is virtually equally effective against many viruses.

Soon after this low-molecular protein was discovered, two main directions of research were formed. The first consisted of searching for inductors, chemical compounds or vaccine-like products, that stimulate the body to independently and intensively produce its own, endogenous interferon. Hundreds of different interferons were tested for this purpose, including synthetic polynucleotides that imitate viruses. This direction of research was headed by foreign scientists, mainly American. At the present time, the most prestigious of them have conceded that all inductors tested thus far are not suitable for use in medical practice.

The second direction of research consisted of developing methods for producing exogenous interferon, a prepared therapeutic agent. Soviet specialists are at the head of this direction. The first laboratory of biosynthesis of interferon was opened in the department of virology of the Institute of Epidemiology and Microbiology (IEM) imeni Honored Academician N. F. Gamaleya, USSR Academy of Medical Sciences, in 1968, and it became the head institution concerned with the state program of investigation and production of interferon.

The product is obtained from human blood leukocyges, since only interferon extracted from human cells is effective. In the last few years, this institute laboratory has produced about 1,800,000 sets of the agent (with five ampules in each set). Later on, at the instigation of IEM, similar production laboratories were opened in Ufa, Perm' and Tbilisi. They have produced almost 8 million sets of interferon.

However, there is a shortage of this protein product, and it is expensive, like most derivatives of human donor blood. For this reason, specialists are continuing to develop new methods of obtaining it. One of them, which has been recognized as an invention, makes it possible to solve the problem of broader production of interferon.

The new and highly effective product meets all of the requirements imposed by our legislation dealing with drugs. At the present time, regulations for industrial production thereof have been approved.

Since interferon enhances general resistance of the body, it would be tempting, of course, to use it for preventive purposes. However, this is not realistic for the time being. More recently, it has been used with success as a therapeutic agent.

Of definite interest is the experience of Prof L. I. Neklyudova, who used this product during the 1969 influenza epidemic. At her instigation, the physicians at Polyclinic No 106 in Oktyabr'skiy Rayon of Moscow gave interferon to flu patients on the first day of illness. There was a decrease to two-fifths in number of days of disability!

Soviet and foreign scientists continue to search for new areas of application of interferon. The first reports have been published on the efficacy of this protein in the control of some oncogenic viruses (which induce development of malignant diseases).

All this explains the heightened interest in methods of producing interferon and studying its properties. The Presidium of the USSR Academy of Medical Sciences adopted a resolution providing for expansion and activation of work on the "Interferon" program with involvement of a number of scientific research institutes and industrial enterprises of our country.

10,657
CSO: 1870

UDC 615.916'126.51.015.43

ACTIVITY OF LYSOSOMAL AND CYTOPLASMIC ENZYMES IN EXPERIMENTAL CARBON DISULFIDE INTOXICATION

Moscow VOPROSY MEDITSINSKOY KHIMII in Russian No 2, 1978 pp 151-156 manuscript received 1 Apr 77

[Article by R. V. Merkup'yeva, L. I. Bushinskaya, B. V. Aulika, I. S. Shaternikova, Ye. I. Protsenko, A. A. Kulygina and N. D. Eksler, of the Institute of General and Communal Hygiene imeni A. N. Sysin, Academy of Medical Sciences USSR, Moscow]

[Text] The activity of lysosomal enzymes and content of biopolymers containing carbohydrate (glycosaminoglycans and glycoproteins) varied in different tissues (liver, kidney, brain and aorta) of experimental animals intoxicated with carbon disulfide. The possible role of the observed impairments, related to neurotropic and hepatotoxic effects, is discussed. Subsequent development of atherogenic and embryotoxic effects of carbon disulfide -- one of the contaminators of the environment -- are considered.

One of the manifestations of toxic effect of chemical contaminants, among them lipotropic substances, and in particular carbon disulfide, can be the direct effect of those compounds or their metabolites on biological membranes. The change of structural integrity and permeability of biological membranes can in turn be one of the main causes of imbalance of various enzymatic systems in the cell.

In spite of a large number of publications in this area, the mechanisms affecting enzyme systems located in different places in the cell under the effect of environmental factors have not yet been definitively explained. Remaining unsolved is the question of the role of enzymes of lysosomal origin and enzymes not bound to cell organelles in the development of direct toxic effect, and also in the origination of a number of remote consequences (embryotoxic, atherogenic, carcinogenic, allergenic and other types of biological effect).

The study of this question presents all the more interest because the change of activity of lysosomal enzymes accomplishing the catabolic phase of

metabolism of a number of biologically important compounds, including carbohydrate-containing biological polymers, proteins, etc [1,2] can contribute to disruptions of exchange of many physiologically important structural elements, for example, brain gangliosides, glycosaminoglycans, glycoproteins, etc. Such disruptions, fraught with unfavorable consequences for the functional state of the cell, can lead to the development of prepathological and morbid phenomena [3,4,5].

A no less important role in the biological effect of environmental factors can also belong to soluble enzyme systems accomplishing the exchange of protein-bound carbohydrates which are a minor component of cell membranes [6,7,8]. Of special interest in that respect is investigating the activity of the enzyme cytosol -- the aldolase of neuraminic acid, which catalyzes the cleavage of free N-acetylneuraminic acid, which together with other monosaccharides (hexosamines, neutral hexoses, fucose, etc) is a membrane-bound carbohydrate and is a structureal component of glycoproteins [9,10].

The present work presents the results of investigation of the activity of enzymes located in the lysosomes and soluble in cytosol, and also the content of carbohydrates bound to proteins on an experimental model of the inhalational effect of a widespread chemical contaminant, carbon disulfide. That compound exerts a well-expressed neurotropic and hepatotropic effect and also contributes to the development of atherogenic, embryotoxic and gonadotoxic effects [11,12,13]. The metabolic mechanisms of the effect of carbon disulfide on the studied enzyme systems of lysosomes and lysosol have not been made clear.

Procedure

The experiment was conducted on 32 rabbits which were subjected for 6 weeks to the effect of carbon disulfide on the level of the threshold dose (0.2 mg/m^3) and at a 10 times larger dose (2 mg/m^3). The biochemical investigation was conducted on the liver, kidneys, brain, aorta and blood serum of the rabbits after 1, 2 and 6 weeks of the effect of carbon disulfide in a high dose and after 6 weeks from the start of its effect in a low concentration.

The activity of enzymes of lysosomal origin: hyaluronidase (KF 3.2.1.21) [14], N-acetyl-β-D-glucosaminidase (KF 3.2.1.30) [15], β-glucosidase (KF 3.2.1.21), β-galactosidase (KF 3.2.1.23) [16] and acid phosphatase (KF 3.2.3.2) [17]. was determined in tissue homogenates. The activity of individual enzymes was determined in fractions of liver lysosomes and the supernatant fraction with the addition of triton X-100 (a final concentration of 0.1%) and without detergent, which permits estimating the degree of strength of the bond of the enzymes with the lysosomal membranes and the effect of labilization [1].

Together with that, the content of hexuronic acids in the tissue were determined [18], which reflects the total quantity of glycosaminoglycans, the

activity of the soluble enzyme hyaloplasm -- the aldolase of N-acetylneuraminic acid [19] and also the content of carbohydrates bound to proteins -- N-acetylneuraminic acid [20], amino sugars and hexoses [21]. The content of N-acetylneuraminic acid and the activity of the aldolase of that acid were determined in different sections of the rabbit brain -- the gray and white matter and the olfactory bulbs. In the blood serum the activities of hyaluronidase and acid phosphatase were investigated, and also the concentration of N-acetylneuraminic acid, hexosamines and hexoses of glycoproteins by the above-indicated methods. The results were processed statistically.

Results and Discussion

Investigation of enzyme systems of lysosomal origin in test rabbits showed that the change of enzymatic activity depends on the type of enzyme, the organ to which it belongs, the dose and the time of effect of carbon disulfide (Figure 1). Very substantial changes were detected on the part of hyaluronidase and N-acetyl-β-D-glucosaminidase, the activity of which in tissue of the liver and kidneys in the early periods of the effect of carbon disulfide diminished, and later increased, in a number of cases exceeding the normal level.

The activity of the acid glycosidases -- β-glucosidase and β-galactosidase, and also of the acid phosphatase changed mainly in the direction of increase, and this was manifested especially noticeably under the influence of a large dose of carbon disulfide (2 mg/m^3) in tissue of the brain and kidneys, where the activity of those enzymes was on the average 2.2-2.4 times that of the control level. An exception is the β-galactosidase of the liver, the activity of which after 6 weeks of effect of carbon disulfide in a small dose (0.2 mg/m^3) was reduced to ten fifty-seventhes in comparison with the control.

The change of activity of lysosomal glycosidases of the liver occurs mainly through free activity (not bound to lysosomes), which gives grounds for assuming the labilization of membranes of lysosomes under the effect of carbon disulfide.

Determination of the content of glycosaminoglycans in the lever of test rabbits showed that the change of the activity of the lysosomal enzymes participating in the reaction of their hydrolytic cleavage was accompanied by accumulation of hexuronic acids, which is especially noticeable in the early periods of effect of carbon disulfide (4.2 times as much; $P < 0.001$).

In frog kidney tissue after 6 weeks of the effect of carbon disulfide a reliable reduction of the content of protein-bound carbohydrates -- hexoses and hexosamines (Figure 2). The accumulation of hexuronic acids (3.5 times as much) was noted in frog aorta tissue after 6 weeks of the effect of carbon disulfide.

The detected changes in the content of carbohydrates bound to proteins and of the activity of lysosomal glycanohydrolases and, consequently, of catabolism

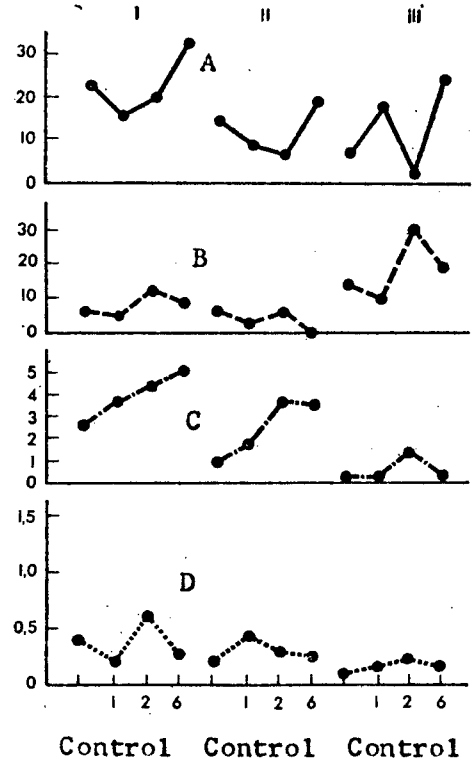

Figure 1. Activity of lysosomal enzymes in frog tissues as a function of the time of effect of carbon disulfide (2 mg/m^3). A -- N-acetyl-β-glucosaminidase (in micrograms of n-nitrophenol per mg of protein per hour); B- hyaluronidase (in micrograms of N-acetylglucosamine per mg of protein in 18 hours); C - β-glucosidase (in micrograms of n-nitrophenol per mg of protein per minute); D - β-galactosidase (in micrograms of n-nitrophenol per mg of protein per minute). I -- liver; II -- kidneys; III -- brain. On axis of abscissas -- period of effect (in weeks); on axis of ordinates - activity of enzymes in above-indicated units.

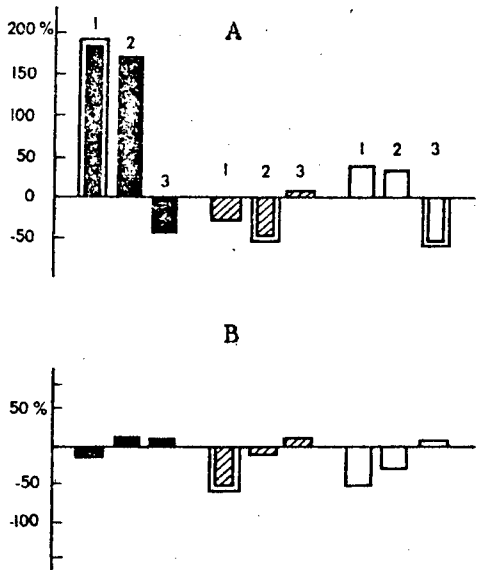

Figure 2. Change of the content of carbohydrates bound to carbons in rat tissues after 6 weeks of inhalation effect of carbon disulfide. Carbon disulfide concentration: A - 2 mg/m^3; B - 0.2 mg/m^3. On axis of coordinates -- change (as % of control): 1 - hexosamines; 2 - hexoses bound to proteins; 3 - N-acetylneuraminic acid. Black columns -- liver; hatched -- kidneys; light -- brain; columns in frame -- statistically reliable changes ($P < 0.05$).

of glycosaminoglycans in the tissue of the brain, kidneys and liver during carbon disulfide intoxication can contribute to disruption of the functional state of the central nervous systems and the organs of detoxication. This applies, in particular, to the excretory function of the kidneys, since at the present time the participation of glycosaminoglycans in the accomplishment of permeability of structures of the medulla renis and processes of water reabsorption has been established [22], and that increase of the concentration of glycosaminoglycans, the metabolism of which is of great importance in assuring the normal activity of the vascular system, can be connected with intensified deposition of those biopolymers in the innermost connective tissue of the vessels, which is typical of the sclerotic changes of the vascular wall and the development of pre-infarction states [23].

It should be noted that in individual periods of effect of carbon disulfide differently directed change of the activity of the functionally connected glycosidases -- hyaluronidase and N-acetyl-β-D-glucosaminidase, when increase of the activity of one enzyme is accompanied by reduction of the activity of the other, was observed in one and the same organ. It can be assumed that the phenomenon, which occurred in the frog liver after 6 weeks, in the kidneys after 2 and 6 weeks and in the brain tissue after 2 weeeks of the effect of carbon disulfide in a large dose (see Figure 1), is evidently an expression of adaptational reactions on the part of the enzyme systems of lysosomes of vitally important organs. The most sensitive to the effect of carbon disulfide of the enzyme system of lysosomes in brain tissue, according to the obtained results, is hyaluronidase, the activity of which after 2 weeks of the effect of carbon disulfide in a large dose was reliably reduced by 68% in comparison with its value in control rabbits. Similar, no less expressed changes were revealed in the same times of experiment in kidney tissues.

Comparative analysis of the obtained data has shown that the change of activity of lysosomal enzymes under the effect of carbon disulfide is accompanied by disruption of the activity of the enzyme system not connected with subcellular structures -- the aldodase of N-acetylneuraminic acid. Very substantial and early changes are typical of the cerebral cortex, in which a simultaneous reduction (35% on the average) of both the activity of the enzyme ($P < 0.01$) and of the content of N-acetylneuraminic acid ($P < 0.001$ already after a week of effect of carbon disulfide in a high concentration. Reliable suppression of the activity of the aldodase of N-acetylneuraminic acid was also observed in more remote periods -- 6 weeks after the start of the experiment (see the table). The discovered disruptions evidently indicate a reduction of intensity of metabolism of N-acetylneuraminic acid in the cerebral cortex under the effect of carbon disulfide.

The presented results testify to the high sensitivity of metabolism of protein-bound carbohydrates, and especially of N-acetylneuraminic acid in brain tissues, to the toxic effect of carbon disulfide. This hypothesis agrees with the information in the literature indicating that the connection of some types of neurotoxins with structures of the central nervous system is

Content of N-acetylneuraminic acid (NA) in the tissues and blood serum and activity of NA aldolase in the tissues and blood serum of rabbits exposed to the effect of carbon disulfide in a concentration of 2 mg/mg^3

a Объект исследования	b Биохимические показатели	c Время воздействия, (нед.) d контроль	1	2	3
1 Печень	a) НК	57±2 (7)	73±7 (4)	83±3 (4)*	67±7 (5)
	b) Альдолаза НК	21±4 (7)	12±2 (4)*	19±3 (4)	12±1 (5)*
2 Обонятельные луковицы мозга	a) НК	97±8 (5)	131±6 (4)*	123±26 (3)	92±2 (4)
	b) Альдолаза НК	17±3 (4)	21±7 (4)	18±4 (3)	14±2 (5)
3 Серое вещество мозга	a) НК	122±2 (13)	92±19 (4)	123±17 (4)	120±4 (5)
	b) Альдолаза НК	18±2 (11)	11±2 (4)*	13±3 (4)	10±2 (5)*
4 Белое вещество мозга	a) НК	122±2 (13)	119±5 (4)	109±6 (4)	107±7 (5)
	b) Альдолаза НК	18±2 (11)	19±4 (4)	9±2 (4)*	13±2 (5)
5 Сыворотка крови	a) НК	71±2 (21)	68±2 (4)	94±4 (7)*	83±5 (5)*

Key: a - Object of investigation b - Biochemical indicators c - Time of effect (weeks) d - Control
1 - Liver 2 - Olfactory bulb 3 - Brain gray matter 4 - Brain white matter 5 - Blood serum
a) N-acetylneuraminic acid (NA) b - NA aldolase

accomplished through the N-acetylneuraminic acid of the brain gangliocides [24]. Also serving as a confirmation of this are the results of a comparative analysis made by us of the results of biochemical and neurophysiological examinations of the brain of experimental animals in studying the primary and secondary responses of the generated potential of the visual cortex of the brain. Thus the reduction of amplitude of the primary response of the generated potential, which is evaluated as increase of excitability of the central nervous system, is accompanied by a reduction of activity of the N-acetylneuraminic acid aldolase in the white matter (after 2 weeks) and in the gray matter of the frog brain (after 6 weeks) during the entry of carbon disulfide into the organism through inhalation (2 mg/m^3). Such a correlation is possibly caused by meurochemical mechanisms of the neurotropic influence of carbon disulfide on the central nervous system.

According to contemporary concepts the change of the architectonics and stability of biological membranes is one of the factors determining the activity of membrane-bound enzymes [23]. The disruption of the activity of acid hydrolases of lysosomal origin established by us and the change of the metabolism of carbohydrate-containing biological polymers in the liver, kidneys and brain of experimental animals can be regarded, in our

opinion, as one of the mechanisms of action of carbon disulfide, which evidently exerts a damaging effect on the integrity and permeability of lysosome membranes. Those disruptions can serve as the material basis of unfavorable remote consequences for the organism as a whole, particularly embryotoxic effect, the development of which under the effect of carbon disulfide has been demonstrated under experimental conditions [13]. It is possible that disturbances of metabolism under the effect of chemical contaminants can be accompanied by the development of predisposition of the organism to the development of certain pathological states, all the more so that at the present time hereditary enzymopathies are known which are caused by a genetic defect of the lysosomal hydrolases studied by us [25]. The potential danger of toxic factors of the environment for man, from the point of view of the possible development of remote consequences [26], brings about a need for further research in this area which will permit more completely deciphering the biological significance of change of the enzyme systems of different cellular localization during the effect of chemical factors of the environment.

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UDC 615.917'558.1.07:616.831/.831-008.939.6'915

BRAIN AND SPINAL CORD GLYCOLIPIDS OF GUINEA PIGS AFTER TRICRESYL PHOSPHATE INTOXICATION

Moscow VOPROSY MEDITSINSKOY KHIMII in Russian No 2, 1978 pp 188-192 manuscript received 21 Mar 77

[Article by N. P. Taranova, Department of Biochemistry, First Leningrad Medical Institute imeni Academician I. P. Pavlov]

[Text] An experimental neuroparalytic form of chronic intoxication with tricresyl phosphate (TCP) was induced in adult guinea pigs by means of single intracutaneous administration of TCP (industrial mixture containing 37% of the ortho-isomer) at a dose of 2.0-2.2 ml/kg of body weight. Moderate and severe forms of the impairment, accompanied by paresis and paralysis of the hind extremities, were developed in 66.1% of the treated animals. The content of galactolipids (cerebrosides + sulfatides) and gangliosides was determined in the brain stem, lumbar and sacral parts of the spinal cord of intact and impaired animals. The content of galactolipids was distinctly reduced in the spinal cord (by 22.9%) and in the brain stem (by 9.0%). Total gangliosides were reduced by 19.1% in the spinal cord, but their content was altered in the brain stem. These alterations appear to reflect destructive processes not only in the myelin membranes but also in the structures of neurones.

A considerable place in the pathology of the nervous system is occupied by demyelinizing diseases as a result of chronic intoxication by organophosphorus compounds widely used in industry and agriculture. A very well-expressed neurotoxic effect, not connected with their anticholinesterase properties, is possessed by diisopropylfluorophosphate, mipafox and tricresyl phosphates, among which the most toxic is triorthocresyl phosphate. They cause specific secondary demyelinization of nerve tissue, which leads to the development of persistent pareses and paralyses of the extremities [1-3].

Since, according to contemporary concepts of the ultrastructure of myelin, the main role in its molecular organization is allotted to lipid groups [4],

in the investigation of the mechanism of the demyelinizing effect of organophosphorus compounds the need to reveal disruptions in the lipid composition of the myelin becomes evident. In spite of the stereotypical character of the clinical picture of poisonings in people, for a long time no experimental model of the neuroparalytic form of intoxication in mammals has existed, and investigations have been carried out mainly on chickens [5-9]. The experimental model of demyelinization caused by tricresyl phosphate in guinea pigs, developed by Eil'ber [2,10] and Dvorkin [11], makes it possible to comprehensively and systematically investigate its damaging effect on the nervous system of mammals.

The task of the present work was the investigation of quantitative changes of the content of galactolipids (cerebrosides + sulfatides) and gangliosides in the brain and spinal cord of guinea pigs during chronic TCP intoxication.

Procedure

The investigations were conducted on mature male and female guinea pigs to whom TCP was administered intracutaneously repeatedly (the TCP was an industrial oil-like mixture containing 37% of the ortho-isomer of TCP) in a dose of 2.0-2.2 ml/kg of mass. Twenty-five to 30 days after the administration of TCP, animals with severe symptoms of neuroparalytic effect were killed and the truncal part of the brain and the lumbosacral section of the spinal cord were removed for biochemical analysis.

Table 1 Estimation of the gravity of clinical manifestations of neurotoxic effect of TCP in guinea pigs

Form of disease	Degree of gravity	Clinical manifestations
Mild (recovering)	+	Poor mobility of rear extremities, ataxia.
	+ +	Well-expressed weakness of the muscles of the rear extremities, difficulty during movement, reduction of the interdigital space, increase of the length of the freely hanging foot, mild pareses of the rear extremities, losses of mass of up to 12-15%
Medium (membrane-toxic)	+++	Weakness of muscles of the rear extremities, interdigital space is missing, length of freely hanging foot increased maximally (up to 45-50 mm), movement difficult, well-expressed pareses of rear extremities, cachexia, loss of 15-20% of weight, rare weakening of tonus of intercostal muscles
Severe (often lethal)	++++	Interdigital space lacking, fingers closed and twisted into a fist, length of freely hanging food maximal, deep pareses and paralyses of rear extremities, losses of mass of up to 20%, intercostal muscles tonus sharply weakened, respiration disrupted.

Lipids were extracted from the tissue homogenate with a mixture of chloroform and methanol (2:1 and 1:2) by Folch's method as modified by Suzuki [12].

The content of gangliosides in the upper aqueous phase after its dialysis against distilled water was determined by the quantity of N-acetylneuraminic acid, using the resorcin method of Svennerholm [13]. The content of galactolipids (cerebrosides + sulfatites) in the lower chloroform phase was determined colorimetrically by the quantity of galactose, using Radin's method [14], but using for the development of a color reaction orcin reagent instead of anthrone (0.1 g of orcin in a cooled mixture of 20 ml H_2O and 50 ml of concentrated H_2SO_4). An additional control for turbidity due to the presence of other lipids was set for each sample. For that purpose an aliquot of the chloroform phase was processed in the same way as the sample, without adding orcin to the color reagent.

Results and Disucssion

The first task of our work was to reproduce the experimental model of the neuroparalytic form of chronic TCP intoxication in which through a single intracutaneous administration of its oil-like technical preparation a "depot" of that substance was created. The gradual lengthy absorption of TCP from the "depot" simulated chronic intoxication. In all the animals during the first 7-8 days after administration of the preparation signs of acute intoxication were observed (deterioration of the general condition, loss of 12-15% of the mass and disruption of functions of the gastrointestinal tract), as a result of which some of the animals died. During the next 2-3 weeks the general condition of the animals improved and their mass was recovered. Twenty-one to 25 days after the administration of TCP, symptoms of its neuroparalytic effect appeared on the background of secondary sharp reduction of the body mass and deterioration of the general condition. Taken as comparable indicators to estimate the degree of myelin damage were clinical tests designated as "interdigital space" and "length of freely hanging foot," which reflect the state of function of the neuromuscular system of the distal and proximal sections of the rear extremities. The degree of gravity of the demyelinizing disease in animals was estimated by criteria developed by Eil'ber [2,10] and Dvorkin [11] with consideration of the neurological symptoms adopted for estimating the degree of myelin damage during other demyelinizing diseases [15].

Data on the reproducibility of experimental demyelinization caused by TCP are presented in Table 2.

Taking into consideration that we observed symptoms of neurotoxic effect of TCP in 84% of the animals, in 66% with medium and severe forms of the disease, we should acknowledge that the reproducibility of the experimental model of the neuroparalytic form of chronic TCP intoxication upon single administration is very great. Moreover, that model is especially valuable for biochemical investigations because it provides the possibility of quantitatively estimating the degree of severity of the disease.

Table 2 Reproducibility of the neuroparalytic form of intoxication in guinea pigs after single intracutaneous administration of TCP (2.0-2.2 ml/kg)

a Общее количество животных	b Летальный исход от острой интоксикации	c Форма заболевания		
		1 легкая (+ и ++)	2 средней тяжести (+++)	3 тяжелая (++++)
62 100%	10 16,1	11 17,7	19 30,6	22 35,5

Key: a - Total number of animals b - Lethal outcome of acute intoxication
d - Form of disease
1 - Mild + and ++ 2 - Medium severe +++ 3 - Severe ++++

Table 3 Content of galactolipids in the brain and spinal cord of guinea pigs in the normal and during chronic TCP intoxication (in micrograms of galactose per gram of tissue)

	a Головной мозг (ствол)	b Спинной мозг (пояснично-крестцовый отдел)
1 Контроль (12)	7,8±0,2	11,8±0,5
2 Трикрезилфосфат (13) (+++ и ++++)	7,1±0,2	9,1±0,4
3 Снижение, %	9,0	22,9
P	<0,02	<0,01

Key: a - Brain (stem) b - Spinal cord (lumbosacral section)
1 - Control (12) 2 - TCP (13) (+++ and ++++) 3 - Reduction, %
Note. Here and in Table 4 the number of animals is given in parentheses.

Table 4 Content of gangliosides in the brain and spinal cord of guinea pigs in the normal and during chronic TCP intoxication (in mg of N-acetylneuraminic acid per gram of tissue)

	a Головной мозг (ствол)	b Спинной мозг (пояснично-крестцовый отдел)
1 Контроль (11)	293±14	173±12
2 Трикрезилфосфат (13) (+++ и ++++)	297±15	140±9
3 Снижение, %	—	19,1
P		<0,05

Key: a - Brain (stem) b - Spinal cord (lumbosacral section)
1 - Control (11) 2 - TCP (13) (+++ and ++++) 3 - Reduction, %

Selected in accordance with the neurological symptoms for biochemical analysis was the most damaged lumbosacral section of the spinal cord, and also the stem part of the brain of animals with medium and severe forms of disease.

The results of determination of the content of total galactolipids (cerebrosides + sulfatides), presented in Table 3, testify that chronic intoxication leads to a distinct reduction (by 22.9%) of the content of those components in the spinal cord specific for myelin. The content of galactolipids in the brain stem also is reduced, but to a considerably lesser degree than in the spinal cord (by 9.0%).

The content of gangliocides, expressed in micrograms of N-acetylneuraminic acid per gram of tissue (Table 4) also is reduced (by 19.1%) in the spinal cord of guinea pigs with medium and severe forms of the disease, but does not change in the brain stem.

In spite of the fact that cerebrosides, according to contemporary concepts, are lipids specific for myelin [4], their content in the nerve tissue during demyelinization caused by organophosphorus compounds has not been investigated up to now. The only research, done on chicks [7], showed that TCP causes a noticeable reduction (by 30%) of the quantity of cerebrosides in the sciatic nerve (calculated on the nerve) and a reduction of the content of the long-chain fatty acids $C_{22:0}$, $C_{24:0}$ and $C_{24:1}$ in them.

The definite reduction of the content of galactolipids detected by us in the spine and brain of guinea pigs during TCP intoxication correlates well with the results of morphological investigations which showed preferential location of damages of the myelin in the spinal cord [2,16], and also the presence of foci of demyelinization in some sections of the brain, in particular in the medulla oblongata of guinea pigs [2].

The results of the investigation permit the assumption that degenerative changes in the structure of the myelin revealed by morphological analysis were caused by disruption of the metabolism of cerebrosides and sulfatides -- necessary components for the formation and maintenance of the structure of the myelin membranes. The reduction of the content of cerebrosides, together with the accumulation of cholesterol esters is typical of demyelinizing diseaes of an autoimmune nature: scattered sclerosis [17,18], experimental allergic encephalomyelitis [19], etc. There are grounds for assuming that loss of cerebrosides is also characteristic of demyelinization caused by other factors, in particular poisoning by organophosphorus compounds, and possibly also by other substances.

We have also investigated the content of gangliocides, which are localized almost exclusively in the gray matter, mainly in neurons, axons and synapses. Gangliosides are closely connected with the functional activity of the central nervous system [20], but they have turned out to be outside the field of vison of researchers studying the neurotoxic effect of TCP and other organophosphorus compounds. We have discovered a reliable reduction of the gangliosides content in the spinal cord during TCP intoxication, although we have not found such changes during allergic demyelinization [19].

Evidently the partial loss of gangliosides in the spinal cord can be regarded not only as biochemical proof of the presence of destructive changes in the neuronal structures, caused by TCP, but also as one of the possible causes of morphological changes of the synaptic and axonal membranes which have been described in chicks [21-23], baboons [24] and primates [25].

If one takes into account the fact of reduction of the content of gangliosides in the rabbit brain by acute organophosphorus intoxication, which was established earlier by us, one can conclude that disruptions in the metabolism of gangliosides are an important element in the pathogenesis of both acute and chronic poisonings with various organophosphorus compounds.

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CSO: 1870

UDC: 616.981.553-092.9-07:616.24-008.4-092-07

CHANGE OF THE HISTAMINE AND SEROTONIN LEVEL IN THE EXTERNAL RESPIRATORY SYSTEM OF CATS WITH BOTULINIC INTOXICATION

Moscow VOPROSY MEDITSINSKOY KHIMII in Russian No 2, 1978 pp 224-226 manuscript received 28 Apr 77

[Article by V. V. Morrison, Department of Pathological Physiology imeni A. A. Bogomolets, Saratov Medical Institute]

[Text] General botulinic intoxication in cats, accompanied by severe respiratory insufficiency, was characterized by distinct impairments of histamine and serotonin metabolism in the external respiratory system. A pronouneed increase in the content of histamine was observed in impaired muscles and their corresponding regional centers of medulla spinalis, when botulinic intoxication was of the local type with unilateral paralysis of the diaphragm and intercostal muscles. The content of serotonin increased in the studied tissues only in the later steps of focal botulinic intoxication.

The clinical picture of botulinic intoxication is characterized by the development of paralysis of the skeletal and primarily the respiratory musculature [1-3]. The paralytic effect of the botulism toxin on motor innervation results from the action of the poison on the motor neurons of the spinal cord [4,5] and blocking of the liberation of acetylcholine with presynaptic formations of cholinergic synapses [6,7].

Starting from the important modulating role of histamine and serotonin in the processes of liberation of acetylcholine in cholinergic synapses [8-12], their influence on processes of eell permeability, electrogenesis [13-16] and the function of respiration [17], it was of interest to clarify how the content of those biologically active substances varies in the external respiratory system during botulinic intoxication.

Procedure

The experiments were conducted on 65 cats with a mass of 2.5-4 kg. Botulin toxic of type C (M.L.D. for the mouse is 0.0006 mg of dry toxin) was

administered intravenously in a dose of 2.2 mg/kg to obtain the general form of intoxication. The investigations were conducted 3 days later on a background of generalized paralytic syndrome. To obtain the local form of intoxication the botulin toxin was administered on one side in the intercostal muscles and diaphragm in a dose of 0.15 mg/kg. Paralysis of only the indicated muscles developed in days. Investigations were conducted in the early (fourth and fifth days) and late (14th and 15th days) stages of botulinic intoxication.

The content of biogenic amines in the tissues was determined spectrofluorometrically: histamine with the use of orthophthalic aldehyde [18] and serotonin with ninhydrin [19].

Results and Discussion

In preliminary tests a study was made of the content of histamine and serotonin in healthy animals, and also in cats 4 days after administration of 0.2 ml of sterile physiological solution in the diaphragm and intercostal muscles. Since in animals of those groups the content of biologically active substances did not differ statistically, they were combined into a single control group.

In general botulism, accompanied by the development of severe respiratory insufficiency, in the experimental animals the content of histamine increased in the intercostal muscles and the cervical and thoracic sections of the spinal cord and its level was reduced in the diaphragm (Table 1).

Together with that an increase was noted in the content of serotonin in the intercostal muscles and the corresponding thoracic segments of the spinal cord. The quantity of serotonin in the diaphragm and cervical section of the spinal cord in the presence of the generalized paralytic syndrome in botulism did not undergo considerable changes in comparison with the control (Table 2).

Thus the development of generalized botulinic intoxication is characterized by well-expressed changes of the content of histamine and serotonin in different tissues of the external respiratory system.

The development of general botulinic intoxication is accompanied by severe respiratory insufficiency and respiratory acidosis [20,21], which, as is known, can play a definite role in the change of content of biogenic amines [22]. Therefore to exclude the role of the hypoxic factor in disrupting the content of histamine and serotonin we studied the content of those substances in the local form of botulinic intoxication, when only unilateral affection of the motor muscles was noted. Taking into consideration the data on the possibility of the spread of botulism toxin along the nerve stems in the spinal cord [23,24], we determined the content of biogenic amines not only in the affected muscles but also in the corresponding segments of the spinal cord.

Table 1 Change of the content of histamine (in micrograms per gram of tissue) in experimental botulinic intoxication in cats

a Исследуемая ткань	b Контроль	c Общий ботулизм	d Местный ботулизм	
			1) ранняя стадия	2) поздняя стадия
a) Диафрагма	0,45±0,049 (19)	0,26±0,035 (10)	0,58±0,035 (20)	0,88±0,091 (10)
P		<0,01	<0,001	<0,001
b) Межреберные мышцы	0,31±0,013 (17)	0,52±0,051 (10)	0,54±0,029 (10)	0,53±0,058 (9)
P		<0,001	<0,001	<0,01
c) Спинной мозг (шейный отдел)	0,25±0,01 (15)	0,74±0,081 (9)	0,34±0,014 (10)	1,09±0,22 (10)
P		<0,001	<0,001	<0,001
d) Спинной мозг (грудной отдел)	0,38±0,017 (12)	0,76±0,046 (9)	0,45±0,019 (10)	1,07±0,201 (10)
P		<0,001	<0,001	<0,001

Table 2 Change of the content of serotonin (in micrograms per gram of tissue) in experimental botulinic intoxication in cats

a Исследуемая ткань	b Контроль	c Общий ботулизм	d Местный ботулизм	
			1) ранняя стадия	2) поздняя стадия
a) Диафрагма	0,95±0,035 (20)	0,83±0,068 (10)	0,71±0,04 (9)	1,17±0,054 (10)
P		>0,1	<0,001	<0,001
b) Межреберные мышцы	0,72±0,033 (20)	0,87±0,031 (10)	0,74±0,034 (10)	1,38±0,047 (10)
P		<0,01	>0,5	<0,001
c) Спинной мозг (шейный отдел)	1,72±0,074 (16)	1,41±0,188 (10)	1,08±0,038 (10)	2,16±0,145 (10)
P		>0,1	<0,001	<0,001
d) Спинной мозг (грудной отдел)	1,5±0,04 (16)	1,8±0,114 (10)	1,14±0,057 (9)	2,32±0,27 (10)
P		<0,01	<0,001	<0,001

Note: In Tables 1 and 2 the number of observations is shown in parentheses; P was calculated in comparison with the control.

Key: a - Investigated tissue; b - Control; c - General botulism; d - Local botulism. 1) early stage; 2) late stage
a) Diaphragm; b) Intercostal muscles; c) Spinal cord (cervical section); d) Spinal cord (thoracic section)

As is evident from Tables 1 and 2, in the early stage of local botulism a distinct increase of the histamine concentration occurs in all the studied tissues. Also noted in that stage is a certain lowering of the serotonin level in the diaphragm and the cervical and thoracic sections of the spinal cord.

More distinct disruptions of histamine metabolism have been detected in the external respiration system in the early stage of local intoxication, when its content increased still more and was fairly high, especially in the nerve tissue. Fourteen or 15 days after the intramuscular administration of botulism toxin in sublethal doses, in contrast with the early stage of intoxication, a considerable accumulation of serotonin was noted in the respiratory muscles and sections of the spinal cord in their region.

In analyzing the obtained data it can be noted that the administration of botulin toxin is accompanied by regular changes of the content of histamine and serotonin in both the general forms of intoxication, complicated by the development of an asphyxial syndrome, and in the local, not accompanied by the development of well-expressed hypoxic hypoxia. The latter gives grounds for thinking that the hypoxic factor does not play a dominant role in the mechanisms of disruption of the metabolism of histamine and serotonin during botulinic intoxication.

Taking into consideration the exceptionally broad spectrum of effect of histamine and serotonin, one can assume that the changes of metabolism of biologically active substances in the nerve and muscle tissue detected by us can have a relation to the disruption of the functional state of the neuromuscular system that occurs in botulinic intoxication.

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PROS AND CONS OF STATIC ELECTRICITY DISCUSSED BY MEDICAL INSTITUTE

Riga SOVETSKAYA LATVIYA in Russian 30 Mar 78 p 3

[Article by F. Portnov, professor, head of the department of clinical biophysics of the Medical Institute of Riga]

[Text] In many areas of the national economy powerful electrical fields are finding more and more use. They are being used for electrofiltration and purification of gases, for electrostatic painting and electric spinning, for electric separation and enrichment of healthful minerals, and for sorting grain. The new technological trend, based on utilization of powerful electrical fields, has been designated electrostatic technology. This technology is raising the productivity of work with a substantial reduction in the cost of production and economization of materials.

However, the introduction into industry and into private life of synthetic materials with high dielectric strength leads to creation and accumulation of electrostatic charges on technical equipment, materials, clothing and surfaces. This causes charge build-up and formation of sizable electrostatic fields, at times disturbing the normal flow of technological processes and leading to ignitions, explosions and injury.

Thus, static electricity appears in certain instances in the capacity of man's friend, enhancing his technological opportunities, and in others in the capacity of a dangerous enemy.

Until recent times the problems of the biological effect of electrostatic fields remained unclear, and scientifically based appreaches to setting rates of maximum permissible levels of this physical factor were lacking. A collective of workers from the clinical biophysics department of the Medical Institute of Riga, one of the first in the country, began comprehensive research into the problem of the biological effect of static electrical fields, development of hygenic standards and recommendations on control of their harmful and dangerous manifestations. The institute has been carrying out experimental research for several years already, analyzing specific conditions of the formation and accumulation of electrostatic charges in various industrial enterprises, and evaluating the